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THE FATE OF BACTERIA INTRODUCED INTO THE UPPER AIR PASSAGES

By Arthur L. Bloomfield

(From the Medical Clinic, The Johns Hopkins University and Hospital.)

In considering the pathogenesis of infectious diseases in which the portal of entry is in the upper respiratory tract, three factors must be reckoned with: first, the means whereby the virus is conveyed to the individual; secondly, the fate of the organism from the time it reaches the mouth or nose until it is eliminated or until invasion takes place; and third, the actual invasion of the virus into the body. Although many details of the mode of conveyance of bacteria from one host to another have been adequately determined, we possess little exact information about the localization, growth and disposal of such organisms, and the factors which determine a longer or shorter period of survival in the upper air passages before or after invasion has taken place. Studies of contact infection and of carrier incidence and persistence under various conditions afford indirect information, which is, however, inadequate to solve the detailed problems of particular infections. By actually introducing bacteria into the upper air passages in such a way that the dose, exact time and site of inoculation, and other conditions can be controlled, and by studying the fate of such organisms, it seems probable that knowledge of the details of the spread of bacteria and of the development of the carrier state can be extended.

It seemed advisable to make a preliminary study of the fate of non-pathogenic organisms before employing possible disease producers. To be satisfactory for this purpose, an organism was sought which was non-pathogenic, not normally present in the upper air passages of man, which could be readily grown on simple media, which could be easily recognized both in culture and in smear, and which would not be overgrown in culture by the normal mouth flora. After examining a number of strains, a sarcina lutea was selected as fulfilling these criteria. The organism employed * had the following characteristics:

Morphology.—Rather small sarcinæ, varying in size, occurring for the most part in regular packets, singly or in pairs, but sometimes in irregular groups. They measure 0.66 to 1.13 microns in 20-hour cultures on plain agar.

Motility.—Non-motile.

Staining Properties.—Gram positive.

Cultural Characteristics.—Agar slant. Growth lemon yellow, at first translucent and rather dull, later, opaque and bright in color, rather thick, raised.

Agar Colonies.—Round with smooth edges, lemon yellow, opaque; surface smooth and glistening. Under low power—margin smooth, sharply defined, surface finely granular, color yellowish grey.

* The culture was kindly supplied by Dr. W. W. Ford.

Broth.—Liquid clouded at first with small amount of viscid sediment; later, liquid clear, with large amount of sediment, very viscid and stringy, yellow, no pellicle.

Peptone Solution.—Less growth than in broth.

Litmus milk.—Unchanged.

Pigment formation often not marked until after the lapse of 48 hours; on very acid or alkaline media, or when grown with certain other organisms, pigment formation may be very slight, but there is always a definite yellowish tint.

The organism grows well in mixed culture with the bacteria usually present in the mouth, and it does not tend to be overgrown except in occasional instances in which a "spreader" rapidly covers the entire plate. The colonies of sarcina lutea grow well side by side with, and at times partly superimposed on, those of the mouth bacteria.

This organism was not found in the air passages of any of the controls; and it is entirely non-pathogenic for human beings.

METHODS

Individuals were selected whose mouths and upper air passages presented no acute disease or abnormality other than those usually encountered, such as pyorrhea, dental caries, adherent tonsils, and minor grades of septal or turbinate deformity. From the point of view of this experiment, they could be regarded as normal. The patients were kept under direct observation during the first hour of the experiment and for the most part took nothing by mouth during this time. The inoculations were always made several hours after eating, when the mouth was free from gross food particles.

Solid masses of a 24-hour growth of sarcina lutea on plain agar were swabbed on the selected site, and cultures taken at regular intervals thereafter. They were made for the most part by scraping the mucous membrane with a heavy platinum loop, 0.5 cm. in diameter, and rubbing up the mucus obtained in this way in a drop of sterile salt solution. This was spread over several agar plates in varying dilutions. It was found that many more organisms were recovered by this method than by the usual swab technique. There was usually a uniform discrete spread of colonies. This point is emphasized because we feel that overgrowth in the plates was eliminated and that we obtained a true representation of the percentage of viable organisms present in the swab. The plates were kept for at least a week and the organisms identified by the appearance of colonies and by smears.

Experiment I.—Sarcina lutea was swabbed on the tongue and cultures were made immediately, and at 10-minute, 1-hour, 24-hour and 48-hour intervals. The results are summarized in Table I.

Summary.—Sarcina lutea swabbed on the tongue disappeared very rapidly. Only a few colonies could be recovered from the site of inoculation after ten minutes, and practically none at the end of one hour. Simultaneous cultures from the pharynx, except in one instance, yielded only a few colonies during the first ten minutes.

Experiment II.—Sarcina lutea was swabbed on the nasal mucosa and cultures made immediately and at 10-minute, 1-hour, 24-hour and 48-hour intervals. The results are summarized in Table II.

Summary.—Sarcina lutea swabbed on the nasal mucosa persisted longer than on the tongue, but in only one of five cases could a few colonies (7) be recovered after 24 hours. In spite of the heavy inoculation, only a few colonies could be obtained in nasopharynx cultures made simultaneously with the nose cultures.

Experiment III.—Sarcina lutea was introduced into tonsil crypts and cultures were made immediately, and after 10-minute, 1-hour, 24-hour and 48-hour intervals. The results are summarized in Table III.

Summary.—Sarcina lutea introduced in large amounts into tonsillar crypts could no longer be recovered after 24 hours, and even at the end of an hour the organisms had disappeared in two out of four cases. There seemed to be no tendency toward the development of a carrier state.

THE MECHANISM FOR DISPOSAL OF BACTERIA IN THE UPPER AIR PASSAGES

Despite the frequent presence of pathogenic bacteria in the upper air passages, both in health and in disease, it is clear that a highly effective mechanism is present in these localities which, except under extraordinary conditions, protects against invasion by foreign organisms. Were this not so, it is obvious that the spread of such organisms would soon become universal among the human race, with an attendant high incidence of disease. The effectiveness of this protective mechanism varies with various bacteria, and with the same bacteria at various times, as illustrated by the epidemiological facts of human disease. The details, however, of its action in particular instances of infection have not been completely worked out. A survey of the literature discloses studies on a number of factors which probably combine in making up the complex protective mechanism of the upper air passages. These factors may be classified as: (1) Mechanical (including the flushing action of secretions with the associated acts of swallowing and the ejection of nasal and mouth secretions); (2) chemical (including the effects of the reaction of the mouth and nose secretions with other possible bacterial inhibitory factors); and (3) biological (including such processes as phagocytosis and the effect of bacteria already present on the invader). These factors can be most conveniently discussed from a topographical standpoint.

The Conjunctivæ.—Maxcy[1] states that the lachrymal secretion has no bactericidal action. Stort[2] found that colon bacilli instilled into the conjunctival sac of rabbits could no longer be recovered in culture after one hour. If the lachrymal duct were tied, the bacteria persisted in the conjunctivæ for a much longer time. He concludes, therefore, that the organisms are swept rapidly into the duct, and that the mechanism for preserving the normal sterility of the eye—at least in the case of the organism studied—was a mechanical one, and not dependent on bacterial inhibitory action of the secretions. Maxcy[1] was able in man to recover *B. prodigiosus* from the nose within five minutes after it had been introduced into the conjunctival sac.

The Nasal Passages.—Although the nasal secretion is normally scant, still the mucous surfaces are constantly moistened. The cilia of the epithelium and the secretion of the mucous membranes and that discharged into the nose from the lachrymal duct create a steady current toward the posterior nares.[3] Furthermore, the configuration of the nasal cavities is such that bacteria and foreign particles are for the most part arrested near the anterior nares. Thus, cultures made in normal individuals yield large numbers of organisms

TABLE I.—FATE OF SARCINA LUTEA SWABBED ON THE TONGUE

Date	Name	Diagnosis and condition	Age	Procedure	Number of colonies of sarcina lutea per plate recovered from tongue and pharynx										Control culture before inoculation		Remarks
					Immediately		After 10 min.		After 1 hr.		After 24 hrs.		After 48 hrs.				
					Tongue	Pharynx	Tongue	Pharynx	Tongue	Pharynx	Tongue	Pharynx	Tongue	Pharynx	Tongue	Pharynx	
June 10.	M.	Cardiac, compensated. Mouth in average condition. Scarred tonsils.	50	½ slant S. L. swabbed on anterior half of tongue.	∞ Plate almost pure S. L.	∞ Plate about ½ S. L.	60	1	8	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	Took nothing by mouth until after 1-hour culture.
June 10.	G.	Tuberculous polyserositis. Mouth clean. Ragged tonsils.	15	½ slant S. L. swabbed on anterior half of tongue.	50	2	6	No S. L.	7	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	Took small amount of liquid between second and third cultures.
June 8.	Mo.	Cardiac, compensated. Mouth in average condition.	60	½ slant S. L. swabbed on anterior half of tongue.	∞ Almost pure plate.	10	150	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	Took small amount of water between second and third cultures.
May 31.	H.	Cardiac, compensated. Mouth in average condition.	30	½ slant S. L. swabbed on anterior half of tongue.	∞ Plate almost pure.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	
May 31.	L.	Cardiac, compensated. Mouth in average condition.	50	½ slant S. L. swabbed on anterior half of tongue.	∞ Plate ⅔ S. L.	3	A few cols. S. L. (confluent).	4	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	
May 31.	D.	Nephritis. Mouth in average condition.	40	½ slant S. L. swabbed on anterior half of tongue.	∞ Plate almost pure.	No S. L.	1	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	Took water between second and third cultures.

TABLE II.—FATE OF SARCINA LUTEA SWABBED ON NASAL MUCOSA

Date	Name	Diagnosis and condition	Age	Procedure	Number of colonies of sarcina lutea per plate recovered from nose and nasopharynx										Control culture before inoculation	Remarks
					Immediately		After 10 min.		After 1 hr.		After 24 hrs.		After 48 hrs.			
					Nose	Nasoph.	Nose	Nasoph.	Nose	Nasoph.	Nose	Nasoph.	Nose	Nasoph.		
June 23.	G.	Abdominal aneurism. Nose and mouth normal.	38	½ agar slant of S. L. swabbed on nasal septum.	∞ About ¼ the plate is S. L.	No S. L.	∞ About ¼ the plate is S. L.	No S. L.	∞ About ¼ the plate is S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	Took nothing by mouth until after the 1-hour culture.
June 23.	H.	Cardiac, compensated. Nose and mouth normal.	60	½ agar slant of S. L. swabbed on nasal septum.	∞ About ¼ the plate is S. L.	No S. L.	∞ About ⅔ the plate is S. L.	6	∞ About ⅔ the plate is S. L.	No S. L.	7	No S. L.	No S. L.	No S. L.	No S. L.	Took nothing by mouth until after the 1-hour culture.
June 21.	T.	Cardiac, compensated. Nose and mouth normal. Scarred tonsils.	55	½ agar slant of S. L. swabbed on nasal septum.	∞ About ¼ of plate is S. L.	No S. L.	2	No S. L.	6	6	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	Drank water after the 10-minute culture.
June 21.	F.	Cardiac, compensated. Nose and mouth normal. Large scarred tonsils.	12	½ agar slant of S. L. swabbed on nasal septum.	∞ About ¼ of plate is S. L.	No S. L.	No S. L.	3	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	Drank water after the 10-minute culture.
June 19.	B.	Psychoneurosis. Nose and mouth normal.	20	½ agar slant of S. L. swabbed on nasal septum.	∞ About ¼ of plate is S. L.	No S. L.	∞ About ¼ of plate is S. L.	No S. L.	10	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	Took nothing by mouth until after the 1-hour culture.

at the nasal orifices and few or none from the deeper nasal passages.[4] Maxey[5] recovered *B. prodigiosus* from the nasopharynx 15 minutes after instilling it into the conjunctiva, and we found sarcina lutea in the pharynx 10 minutes after swabbing it on the anterior part of the septum.

With regard to the bacterial inhibitory effect of the nasal secretions, the literature is contradictory, some writers finding a marked inhibitory action and others failing to do so. Variations in the method of collecting and diluting the secretions, in the organism used, and in the method of plating, may account for these discrepancies. Kligler[6] has shown recently that small amounts of nasal secretions added to culture media at times promoted the growth of organisms such as meningococci. He believes this action to be due to "vitamins." The experiments of Calvino,[6] however, which approximate most closely natural conditions, indicate that the nasal mucosa is an unfavorable medium at least for some types of organism.

It seems clear, therefore, that the normal tendency is to preserve sterility of the deeper nasal passages and that no true permanent nasal flora exists.

The Mouth and Throat.—The mouth affords an especially favorable opportunity for some types of bacterial growth; food particles, carious teeth, desquamated epithelium and saliva furnish media whose effectiveness is attested by the enormous numbers of bacteria constantly present even in so-called clean and well-kept mouths. There is, however, a continuous shift in the "normal" flora, so that cultures made from the same individual on successive days may yield different quantitative and qualitative results. Such variations are to be expected with changing food and fluid intake and variations in mouth hygiene. Furthermore, the abundant salivary and mouth secretions with the constant flushing effect of ingested fluids, swallowing and expectoration, undoubtedly constitute a cleansing mechanism.

With regard to the bacterial inhibitory effect of saliva and mouth secretions in *vivo*, no satisfactory data are at hand, although numerous test-tube experiments are on record. Thus, Sanarelli[7] found that streptococci, staphylococci, M. tetragenus and cholera spirilla were rapidly destroyed by fresh filtered saliva *in vitro*, unless an enormous number of organisms were used. The pneumococcus, on the other hand, throve in saliva better than in broth. Barnes[8] using a similar method, found that saliva had no bactericidal action on streptococci or pneumococci. Variations in the results of such experiments are to be expected under various experimental conditions.

The importance of the normal mouth flora in over-growing and suppressing invading pathogenic organisms is indicated only in an indirect way. On artificial media, meningococci, pneumococci and influenza bacilli grow well in certain proportions with the normal mouth organisms. They may, however, be rapidly overgrown by "spreaders," particularly of the aërobic spore-bearing types. It is possible that such organisms may actually exert a similar effect in the mouth, although this is not directly demonstrated.

It is clear, therefore, that a complex mechanism exists in the upper air passages, which under certain conditions suffices to dispose of invading pathogenic microörganisms. The details of its operations are obviously variable, and as is to be expected, it often breaks down. Thus, such factors as dosage of the organism, local pathological processes or anatomical anomalies, variations in the biological activities of the organisms themselves, may play a part in explaining why certain bacteria may gain a foothold at one time; and at another, or in a different individual, be promptly wiped out. From the standpoint of epidemiology it seems more profitable, therefore, to observe the actual fate of organisms introduced into the upper air passages than to try to analyze their exact method of disposal.

TABLE III.—FATE OF SARCINA LUTEA INTRODUCED INTO TONSILLAR CRYPTS

Date	Name	Diagnosis and condition	Age	Procedure	Number of colonies per plate of sarcina lutea recovered from tonsil crypt					Control culture before inoculation	Remarks
					Immediately	After 10 min.	After 1 hour	After 24 hours	After 48 hours		
June 26.	C.	Syphilis. Nose and mouth essentially normal. Small, clean tonsils with sealed crypts.	35	½ agar slant of S. L. introduced into a tonsil crypt.	∞ (Almost pure plate.)	∞ (Almost pure plate.)	∞ (Almost pure plate.)	No S. L.	No S. L.	No S. L.	Took nothing by mouth until after the 1-hour culture.
June 26.	F.	Hodgkin's disease. Large, ragged tonsils.	30	½ agar slant of S. L. introduced into a tonsil crypt.	∞ (Almost pure plate.)	∞ (Almost pure plate.)	6	No S. L.	No S. L.	No S. L.	Took nothing by mouth until after the 1-hour culture.
June 25.	M.	Cardiac, compensated. Large, ragged tonsils with open crypts.	60	½ agar slant of S. L. introduced into a tonsil crypt.	8	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	Took nothing by mouth until after the 1-hour culture.
June 25.	Fi.	Tuberculous pericarditis. Large, scarred adherent tonsils.	15	½ agar slant of S. L. introduced into a tonsil crypt.	∞ (Almost pure plate.)	No S. L.	No S. L.	No S. L.	No S. L.	No S. L.	Took nothing by mouth until after the 1-hour culture.

None the less, an attempt was made to determine, at least approximately, the mechanism which led to the rapid disappearance of sarcina lutea. The reaction of the mouth secretions, the effect of mechanical cleansing, and bacterial inhibitory effects of the mouth secretions were studied.

1. *Effect of the Reaction of Mouth Secretions.*—Saliva and mouth secretions were collected from each of the patients inoculated with sarcina lutea. The mucous membranes were scraped with a large platinum loop, after which the patient expectorated the saliva which had accumulated in the mouth. The entire mixture was diluted with an equal amount of fresh, sterile distilled water, and the reaction immediately tested. The colorimetric comparison method, as now generally used in bacteriological work [9] was employed with phenolsulphonephthalein and alizarin as indicators. The pH of the various specimens is indicated in the following table (Table IV).

TABLE IV

PH OF SPECIMENS OF SALIVA FROM INDIVIDUALS INOCULATED WITH SARCINA LUTEA

Name	pH of saliva immediately	pH after standing one hour
Mo.	5.9	5.9
G.	5.9	—
H.	5.9	—
F.	6.0	6.0
Mu.	6.0	—
L.	7.0	6.9
F.	7.0	7.0
D.	7.2	7.1
T.	7.2	7.0
Ho.	7.3	—
Gr.	7.4	7.1
Fi.	7.6	7.3
Mo.	7.6	—

The range of growth of sarcina lutea was next tested. Tubes of plain agar with reaction adjusted from pH 5.0— to 8.0+ were inoculated. Sarcina grew with equal luxuriance on all the tubes within this range. It seems clear, therefore, that the reaction of the mouth secretions cannot alone account for the disposal of sarcina lutea.

2. *Mechanical Influences.*—An attempt was made to ascertain to what extent the normal washing processes in the mouth serve to remove organisms present. The mouth was thoroughly rinsed with one liter of sterile salt solution. The patient coöperated in making this washing effective by churning the fluid in the mouth and scraping the tongue with the teeth. Such an irrigation would be expected to remove more organisms than the ordinary acts of swallowing and expectoration. The tongue was scraped with a large platinum loop just before and just after the washing and at subsequent intervals. The loop of scrapings was diluted and plated in a standard manner. In no case was it possible to reduce the bacterial count by more than 50 per cent, even by such exten-

TABLE V

BACTERIAL COUNTS FROM TONGUE BEFORE AND AFTER IRRIGATING MOUTH WITH SALT SOLUTION

Name	No. cols. from 1 loop of tongue scrapings before irrigation	No. cols. from 1 loop of tongue scrapings immediately after irrigation	No. cols. from 1 loop of tongue scrapings 10 min. after mouth irrigation
B.	1600	800	∞
M.	∞	∞ Plate contains about as many colonies as before.	∞

TABLE VI.—EFFECT OF SALIVA ON THE GROWTH OF SARCINA LUTEA

One standard loop from each tube plated at following intervals	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7
	0.2 c. c. saliva	0.2 c. c. suspension S. L. + 0.2 c. c. salt solution	0.2 c. c. suspension of mixed mouth bacteria + 0.2 c. c. suspension S. L.	0.2 c. c. saliva + 0.2 c. c. suspension S. L.	0.2 c. c. saliva + 0.1 c. c. suspension S. L.	0.2 c. c. saliva + 0.05 c. c. suspension S. L.	0.2 c. c. saliva + 0.025 c. c. suspension S. L.
Immediately	About 500 cols. of various kinds.	∞ cols. S. L.	∞ cols. S. L. with about 50 cols. mouth bacteria.	∞ cols. S. L. with a few mouth bacteria.	∞ cols. S. L. with a few mouth bacteria.	250 cols. S. L. Mouth bacteria.	100 cols. S. L. Mouth bacteria.
15 minutes	About 300 cols. of various kinds.	∞ cols. S. L.	∞ cols. S. L. with about 50 cols. mouth bacteria.	About ½ as many colonies S. L. as in previous plate, with a good many mouth bacteria.	10 cols. S. L. (appeared after 48 hours). Mouth bacteria.	No S. L. Mouth bacteria.	1 col. S. L. (appeared after 4 days). Mouth bacteria.
1 hour	About 500 cols. of various kinds.	∞ cols. S. L.	∞ cols. S. L. with about 100 cols. mouth bacteria.	2 cols. S. L. (appeared after 48 hours), with a good many mouth bacteria.	1 col. S. L. (appeared after 4 days). Mouth bacteria.	No S. L. Mouth bacteria.	7 cols. S. L. (appeared after 3 days).
2 hours	About 200 cols. of various kinds.	∞ cols. S. L.	∞ cols. S. L. with about 100 cols. mouth bacteria.	No S. L. Mouth bacteria.	No S. L. Mouth bacteria.	No S. L. Mouth bacteria.	No S. L. Mouth bacteria.
24 hours	About 500 cols. of various kinds.	∞ cols. S. L.	∞ cols. S. L. with about 100 cols. mouth bacteria.	No S. L. Mouth bacteria.	No S. L. Mouth bacteria.	No S. L. Mouth bacteria.	No S. L. Mouth bacteria.

sive irrigation. It seems, therefore, that mechanical processes alone cannot adequately account for the rapid disappearance of sarcina lutea. Table V shows an illustrative protocol.

3. *The Inhibitory Effect Upon Bacteria Exerted by the Mouth Secretions and Saliva.*—An attempt was made to reproduce the conditions present in the mouth as nearly as possible by the following method. 0.2 c. c. of fresh saliva was placed in each of a series of tubes. To each was added decreasing amounts of a thick suspension of sarcina lutea. One standard loop of the mixture of saliva and sarcina lutea was removed from each tube immediately and at various intervals, and plated on plain agar plates. The results which coincided in numerous experiments with various specimens of saliva, except as to slight quantitative variations, are illustrated by the following typical protocol (Table VI).

From this protocol, the following facts are apparent:

1. Cultures made from a specimen of saliva at various intervals up to 24 hours yielded about the same number and kind of organisms.

2. Cultures made at various intervals up to 24 hours from a suspension of sarcina lutea in salt solution yielded the same number of colonies.

3. Cultures made at various intervals up to 24 hours from a mixed suspension of mouth flora and of sarcina lutea yielded the various organisms in about the same proportions. Sarcina lutea was not overgrown or inhibited.

4. Cultures made at intervals up to 24 hours from suspensions of varying concentration of sarcina lutea in saliva yielded rapidly decreasing numbers of colonies of sarcina lutea. The number was much reduced in 15 minutes, only an occasional organism could be recovered after 1 hour, and none after 2 hours. Furthermore, the growth of the few organisms obtained after the first culture was inhibited for intervals of 1 to 4 days before the colonies appeared in the plates.

Smears were made from each tube in the above experiment at 15-minute, 1-hour and 2-hour intervals. After 15 minutes, abundant sarcinæ were seen in all tubes. They were normal in staining qualities and in grouping. After 1 hour the smears from the mixtures of saliva and sarcina lutea showed the sarcinæ to stain poorly and to be pale and granular. After 2 hours few definite organisms could be seen. No agglutination was apparent. The organisms from the control tubes stained normally.

It may be concluded, therefore, that saliva exerts a rapid bactericidal effect on this organism and that this effect is independent of the bacteria normally present in the saliva.

DISCUSSION

The general result of these experiments indicates that even after a short period of time it is usually impossible to recover sarcina lutea swabbed in large amounts on the tongue, nasal mucosa, or into the crypts of the tonsils. Disappearance from the nose was somewhat slower than from the other sites; in only one instance could any organisms be recovered after 24 hours, and in no case after two days. Cultures made in this way do not, of course, prove the complete absence of the organism in the mouth and nose, but the general trend of the quantitative relations indicates a rapid disappearance. The fact that the estimated dose of 50 to 100 billion organisms was vastly greater than that in any natural infection, indicates the remarkable efficiency of the mechanism present in the upper air passages for disposing of this organism. An analysis of the possible factors active in effecting this disposal indicated that reaction of mouth secretions, mechanical cleansing, and the mouth bacteria play little if any part, but that the saliva and mouth secretions exert a prompt and marked bactericidal effect.

Similar methods are being used in studying the fate of other organisms introduced into the upper air passages.

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THE INFLUENZA BACILLUS IN PARANASAL SINUS INFECTIONS

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There is some difference of opinion as to the nature of the organism primarily responsible for the influenzal epidemic of 1918-1919. Pfeiffer's bacillus was found in a sufficient number of cases to raise the question as to whether it was the primary agent, or, like the streptococcus and pneumococcus, a secondary invader.

We were interested to determine whether or not this bacillus occurred more frequently in a series of infected nasal sinuses observed immediately following the epidemic, than in a series of such cases observed during a period when there was no epidemic. During the height of the epidemic in Baltimore, it was impossible, owing to the press of work, and the scarcity of skilled help, to make bacteriological examinations of the infected sinuses. Within six weeks after the epidemic had subsided, however, one of us (Neville) undertook a careful bacteriological study of every case of maxillary sinus infection treated at The Johns Hopkins Hospital. We limited our study to the maxillary sinus, because this cavity is the

one most frequently affected, and because the material for culture can be obtained from this situation with less risk of contamination than from the ethmoidal, frontal or sphenoidal cells.

We present for comparison two series of cases. The first series of seventy includes every case of accessory nasal sinus infection treated at The Johns Hopkins Hospital during the years 1912-1918, in which we have a report on the cultures made from the antrum.* Of these 70 observations, made during a period when there was no epidemic of influenza, we have 15 cases in which the influenza bacillus was found in the antrum. The 30 cases of the second series were all observed during February, March and April of 1919. In eight of these cases we found *B. influenzæ* in the antrum. It is of interest that the percentage of cases in which the influenza bacillus was found in the antrum is about the same in each group.

The material for culture is obtained by a very simple method. A small piece of cotton on the end of a steel applicator is moistened with a 20 per cent solution of cocaine and 1:1000 adrenalin. The excess of cocaine is removed by pressing the cotton between two layers of gauze; this will insure against symptoms of cocaine poisoning. A small piece of cotton moistened with cocaine and adrenalin is also placed between the anterior end of the inferior turbinate and the septum, and both are left in place for ten or fifteen minutes. A curved trochar, a small rubber ear syringe, a nasal speculum and a finger bowl are then boiled for at least five minutes. The patient's nose is cleansed of all visible discharge. The trochar is passed under the anterior end of the inferior turbinate into the antrum, and the stilette removed. The patient bends 90° forward, and often the pus will drop from the cannula. If not, air or a few drops of sterile salt solution are blown into the antrum with the rubber syringe, and the material returning through the cannula is caught in a sterile test-tube. It is important that enough adrenalin be used to make the procedure absolutely bloodless, for we have found, in agreement with Rivers, that a few drops of blood mixed with the material obtained for culture will inhibit the growth of the influenza bacillus.[1]

The bacteriological technique employed is as follows: The material is plated immediately on agar containing defibrinated rabbit's blood, and the plates are incubated at 37° C. for twenty-four hours. The various colonies are then examined in stained smears and transferred to blood-agar slants. After twenty-four hours the colonies showing the cultural characteristics of the streptococcus and pneumococcus are differentiated by Neufeld's bile soluble test. The pneumococci are grouped according to the method of Avery, Chickering, Cole and Dochez.[2] The serum used was obtained from the Rockefeller Institute. The influenza bacillus grows freely on blood-agar but not at all on plain agar. This differentiates it from the micrococcus catarrhalis. This latter organism may be differentiated from other gram-negative cocci by the use of Hiss's serum sugar media, and the Elser and Huntoon classification.

In the first series of 70 cases observed during the period from 1912 to 1918, the influenza bacillus was found in the antrum in 15 cases (21 per cent). The streptococcus was found in 33 cases (47 per cent); in 10 of these the organism was hæmolytic. The pneumococcus occurred 12 times (17 per cent). The staphylococcus was found in 16 cases (22 per cent), in one of which it was hæmolytic; the proteus was found once; and the diphtheria bacillus twice.

In the second series of 30 cases observed during the three months immediately following the subsidence of the influenzal epidemic, the influenza bacillus was found in the antrum in eight cases (26 per cent). The streptococcus was again the predominating organism (14 cases, or 46 per cent); of these 14 cases, in four it was hæmolytic. The following organisms were occasionally found: the hæmolytic staphylococcus in four cases, the pneumococcus in three cases, the micrococcus catarrhalis in two cases, and a gram-negative diphtheroid bacillus in three cases. The diphtheria bacillus was found in one case, the bacillus lactis ærogenes once, and the proteus vulgaris in two cases.

The influenza bacillus was recovered from the antrum in pure culture in four cases. In four other cases it was found associated with other organisms: streptococcus hæmolyticus, streptococcus mucosus, micrococcus catarrhalis, micrococcus tetragenus, and the staphylococcus albus. The hæmolytic staphylococcus occurred four times, once in pure culture. The staphylococcus albus was found in eight cases, but was probably in each case a contamination from the nasal cavity.† The pneumococcus was recovered in three cases (in two Type III and in one Type IV), twice in pure culture, once mixed with a staphylococcus albus.

* It was formerly our custom to take cultures from infected sinuses (chiefly at operation) on cotton swabs. These swabs were sent to the regular bacteriological laboratory of the hospital but had often completely dried up before reaching the laboratory. This accounts for the comparatively small number in which we have a bacteriological report.

† The healthy nasal cavity is apparently a normal habitat for the staphylococcus albus. We took cultures from the middle turbinate in seven individuals with clinically normal nasal cavities. A little mucus was obtained from the middle turbinate with a platinum loop and implanted on plates of rabbit blood-agar. We found: staphylococcus albus 91 per cent; diphtheroids, 41 per cent; pneumococcus, Type IV, 16 per cent; micrococcus catarrhalis, 8.9 per cent.

Park and Wright, in 1898, took cultures with a platinum loop from far back in the nose and showed that the nasal secretion has no bacteriocidal action. Of 36 examinations only six were sterile. The predominating organism was the staphylococcus.

Hasslauer[3] in 1902, made bacteriological examinations from the normal nasal cavities of 111 individuals. He found: staphylococcus in 25 per cent; pneumococcus in 20 per cent; streptococcus in 17 per cent; diphtheroid in 13 per cent.

He also investigated the bacteriological content of the nasal discharge in 78 cases with a coryza and found much the same varieties of organisms in both healthy and pathological nasal cavities. The only difference was that they were far more numerous in infected nasal cavities.

CONCLUSIONS

1. The influenza bacillus was found in infected maxillary sinuses in practically the same percentage (21 per cent) in a series of cases observed during a period of six years when there was no epidemic, as in a second series observed immediately following the epidemic in Baltimore during the winter of 1918-1919 (26 per cent).

2. The influenza bacillus is not infrequently found in pure culture in the antrum.

3. Clinically *B. influenzæ* is a pyogenic organism.

4. The predominating organism in all antrum infections is the streptococcus.

5. We infer from these observations that the influenza bacillus, like the streptococcus and pneumococcus, is a secondary invader, and not the primary cause of the disease known as influenza.

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MENINGOCOCCUS PNEUMONIA. I

THE OCCURRENCE OF POST-INFLUENZAL PNEUMONIA IN WHICH THE DIPLOCOCCUS INTRACELLULARIS MENINGITIDIS WAS ISOLATED. FROM OBSERVATIONS AT CAMP COETQUIDON, A. E. F., FRANCE

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Whatever may be the cause of primary influenza, it would seem that the secondary pneumonia may be caused by different organisms in different regions. As MacCallum [1] has pointed out, this variation "may depend on an endemic or epidemic distribution of organisms as inhabitants of the nasopharynx." Pneumococci,[1] streptococci [1] and staphylococci [2] have been the most frequent secondary invaders and evidence now appears [3, 4, 5, 6, 7, 8] that the diplococcus intracellularis meningitidis (Weichselbaum) may produce fatal pneumonia. The cases reported in this paper were studied at Camp Hospital No. 15, Camp Coetquidon, Base Section No. 1, A. E. F., France, during the epidemic of influenza from September 1, 1918, to February 15, 1919.* Table I shows the total number of cases of influenza, pneumonia and cerebrospinal meningitis admitted to the hospital during this period.

Prior to the outbreak of the epidemic of influenza, there were no cases of meningococcus meningitis in the hospital. It will be noted that coincident with the occurrence of meningococcus pneumonia, there occurred a considerable number of meningococcus meningitis cases.

The presence of influenza at Camp Coetquidon was first noted about September 1, 1918, and many cases of pneumonia

* Prior to September 1, 1918, there had been but three deaths in this hospital due to the epidemic. They occurred in the last week of August, 1918. Cultures from the lung tissue at autopsy showed *B. influenzæ*, *pneumococcus* Type III and *pneumococcus* Type II, respectively. They are not included in this study because other data for comparison are not available. This study was terminated on February 15, 1919, because the evacuation of troops, begun at that time, rendered records for comparison unreliable and difficult to secure. On February 15, 1919, there were still eight cases in the hospital diagnosed as meningococcus pneumonia. These are included among the total cases recorded but their final disposition is not included.

Camp Coetquidon was an artillery training area occupied by troops which arrived from the United States during the latter part of August, September and October, 1918, and remained until February 15, 1919. These together with the permanent camp personnel numbered approximately as follows: September, 16,657; October, 19,177; November, 16,303; December, 12,850; January, 13,980; February, 14,780.

developed. On September 3, 1918, bacteriological cultures at autopsy from the lungs of a case of pneumonia showed a pure growth of meningococci (Type A Pasteur). A description of the bacteriological and serological technique employed is appended. On September 6, 1918, a similar case showed meningococcus Type B in the lung culture. Both cases occurred early in the epidemic, the patients were in the same ward at the same time, but the fact that different types of meningococci were isolated would seem to preclude the possibility of either of the two cases having been infected from the other or that both were infected from the same source.

On September 13 and 15 meningococci were recovered from the sputa of two pneumonia cases.

TABLE I

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb. (1)	Totals
Cases of influenza admitted to hospital.	847	263	170	38	80	112	1510
Total cases of pneumonia admitted to hospital.	156	107	53	21	36	30	403
Total deaths from pneumonia.	27	30	12	0	3	6	78
Cases of pneumonia showing meningococci in sputum.	20	15	27	2	13	8	85
Deaths from pneumonia showing meningococci in lungs at autopsy.	6	5	6	0	1	5	23
Cases of meningococcus meningitis.	4	7	7	3	1	0	22
Deaths from meningococcus meningitis.	4	2	3	3	1	0	13

EXPLANATION OF TABLE I

Total cases of pneumonia include pneumonias due to all causes. Many cases admitted as influenza developed pneumonia after admission. Such cases are recorded as pneumonia and not influenza.

Cases of pneumonia showing meningococci are included among the total cases of pneumonia. This group, however, does not include any of the cases in which cerebrospinal meningitis was present.

Total deaths from pneumonia include all deaths from pneumonia. The deaths from pneumonia showing meningococci are included in this group.

Cases of meningococcus meningitis include all cases of cerebrospinal meningitis caused by meningococci, with or without pneumonia involvement.

It seemed, then, that the possibility of the meningococcus being an important factor in the epidemic was worthy of careful investigation. Cultures were therefore taken (September 19, 1918) from the throats of all respiratory cases in the hospital. Among 114 thus cultured meningococci (Type B Pasteur) were isolated from seven patients, six of whom had pneumonia.

On September 20, 22, 23, 25, 27 and 29 throat and sputum cultures of meningococci were recovered from 12 additional cases of pneumonia.

During the period studied, 78 cases of pneumonia came to autopsy. Cultures on blood agar were made from sections of the various parts of the affected lungs in all cases. The findings are of interest here for the purposes of comparing the frequency and the association of the various species of bacteria found during the epidemic. Meningococci were recovered from the lung tissue in a total of 23 cases (in pure culture in 7 cases and in mixed culture in 16). The complete bacteriological results in these 78 pneumonia autopsies are recorded in Table II.

The majority of the meningococci isolated were Pasteur Type B and were also agglutinated by Gordon's meningococcus sera, Types II and IV (corresponding to the Rockefeller Institute normal meningococcus). The cultural and serological findings in these cultures were confirmed at Base Laboratory No. 1 at St. Nazaire and at the Central Medical Laboratory at Dijon.*

In our experience, despite certain contradictory statements in the literature in regard to the correlation of the American, English and French classification of meningococcus types. Lieut.-Col. Gordon's Types I and III and the Rockefeller

TABLE II

BACTERIA ISOLATED FROM THE LUNGS AT AUTOPSY FROM 78 CASES OF PNEUMONIA AT CAMP HOSPITAL NO. 15, A. E. F., FRANCE

B. influenzæ Pure cultures 7 cases, *Mixed " 23 " , associated with Total " 30 " ,	Meningococcus...11 times Pneumococcus IV 7 times Strept. hemolytic. 6 times Strept. vir..... 1 time B. tuberculosis... 1 time
Pneumococci IV Pure cultures 11 cases, *Mixed " 14 " , associated with Total " 25 " ,	B. influenza..... 7 times Strept. hemol.... 4 times Meningococcus .. 3 times
Meningococci Pure cultures 7 cases, Mixed " 16 " , associated with Total " 23 " ,	B. influenza.....11 times Strept. hemol.... 6 times Pneumococcus IV 7 times Strept. vir..... 1 time B. tuberculosis... 1 time
Streptococcus hemolyticus Pure cultures 9 cases, Mixed " 11 " , associated with Total " 20 " ,	B. influenza..... 6 times Pneumococci IV.. 4 times Meningococci .... 2 times B. tuberculosis... 2 times
B. tuberculosis Pure 0 cases, Mixed 6 " , associated with..... Total 6 " ,	Staphylococcus... 2 times Strept. hemolyt.. 2 times Strept. vir..... 1 time B. influenzæ ..... 1 time
Streptococcus viridans.....2 cases Staphylococcus aureus.....2 " Pneumococcus I .....1 case Streptococcus mucosus.....1 " Bacillus aerogenes capsulatus...1 " Staphylococcus albus .....1 "	Associated with other organisms as indicated above

*Mixed cultures refer to two or more species of bacteria

Institute paranormal sera usually agglutinate the same cultures as Pasteur Type A. Colonel Gordon's sera II and IV and the Rockefeller Institute normal meningococcus serum usually agglutinate the same cultures as Pasteur Type B. Pasteur Types C and D are rare.

Gram-stained smears of the sputa from the pneumonia cases in which meningococci were isolated showed so many intracellular gram-negative diplococci as to resemble smears of spinal fluids from acute cases of meningococcus meningitis.

CLINICAL COURSE OF MENINGOCOCCUS PNEUMONIA

In the severe cases the onset was usually acute, after a period of indisposition lasting from a few days to a week. During the prodromal period there was generally a history of chills,

* Thanks are due to Captain E. H. Mason, M. C., U. S. Army, for assistance in confirming these cultures.

TABLE III

Case	Organization	Days in Hosp.	Laboratory findings during illness				Date of death	Autopsy findings		Autopsy number
			Urine	W. B. C.	Blood cultures.	Sputum cultures		Lung gross anatomy	† Lung cultures	
V. F.	133 d F. A.	2	Alb. and casts	6400	Neg.	Mixed	9/3	Lobar pneumonia	Meningo. A.	25
T. R.	44th Engrs.	5	Alb. and casts	4400	Neg.	B. inf.	9/6	Bronchopneumonia	Meningo. B.	28
J. B.	141st F. A.	12	....	7000	Neg.	Meningo. B.	9/21	Bronchopneumonia	Meningo. B.	36
S. C.	114th T.M.B.	6	....	13500	Neg.	Pneumo IV.	9/22	Bronchopneumonia	Meningo. B. Pneum. IV.	38
J. O'N.	61st Engrs.	7	....	7600	Neg.	B. inf.	9/25	Lobar pneumonia. Sup.* bronchiolitis.	Meningo. B.	44
C. S.	141st F. A.	2	....	....	....	....	9/29	Lobar pneumonia	Meningo. B. Pneum. IV,	49
A. C.	141st F. A.	17	....	4400	Neg.	Meningo. B. diphtheroid.	10/3	Bronchopneumonia. Sup. bronchiolitis.	B. inf. Meningo. B.	57
M. D.	141st A. T.	7	....	9600	Neg.	Meningo. B.	10/3	Lobar pneumonia	Meningo. B. Strept. vir.	58
R. S.	329th F. A.	6	....	9400	Neg.	Meningo. B.	10/8	Lobar pneumonia	Meningo. B.	66
W. C.	329th F. A.	11	....	6200 9000	Neg.	B. inf. Pneu. IV.	10/24	Bronchopneumonia. Sup. bronchiolitis.	B. inf. Meningo. B.	86
R. C.	328th F. A.	2	Alb. and casts	5400	Neg.	B. inf.	10/27	Bronchopneumonia	B. inf. Meningo. B.	89
F. N.	329th F. A.	3	....	4200	Neg.	B. inf. Pneu. IV.	11/2	Bronchopneumonia. Wet lung.	B. inf. Meningo. A.	95
B. P.	Vet. Hosp.	3	Alb. and casts	2200	Neg.	B. inf.	11/3	Bronchopneumonia. Wet lung.	B. inf. Meningo.	98
L. F.	330th F. A.	9	Alb. and casts	2600 4200	Neg.	B. inf.	11/4	Bronchopneumonia.	B. inf. Meningo. C.	100
O. A.	113th A. T.	8	Alb. and casts	18400	Neg.	Meningo	11/9	Bronchopneumonia. Sup. bronchiolitis.	Meningo. Strept. hemol.	105
E. N.	Vet. Hosp.	11	Alb. and casts	8000	Neg.	Meningo	11/13	Bronchopneumonia. Sup. bronchiolitis.	B. inf. Meningo.	106
A. P.	131st F. A.	13	Alb. and casts	9200 20,400	Neg.	B. inf. Meningo.	11/23	Empyema. Bronchopneumonia. Sup. bronchiolitis.	B. inf. Meningo. Strept. hemol.	111
C. G.	133d F. A.	9	Alb. and casts	2200	Neg.	Meningo. B.	1/29	Bronchopneumonia. Sup. bronchiolitis.	Meningo. B.	124
G. S.	133d F. A.	6	Alb. and casts	11,200 25,000	Neg.	Pneu. IV. Meningo.	2/1	Bronchopneumonia. Sup. bronchiolitis.	Meningo. B. Pneu. IV.	125
A. J.	7th Vet. Hosp.	8	Alb. and casts	5200 9600	Neg.	B. inf. Meningococcus	2/1	Bronchopneumonia. Sup. bronchiolitis.	Meningo. B.	127
J. D.	132d F. A.	3	Alb. and casts	7200	Neg.	Meningo. B.	2/3	Bronchopneumonia. Sup. bronchiolitis.	Meningo. B. B. inf.	128
D. K.	705th M.T.C.	6	Alb. and casts	1400 4400	Neg.	Meningococcus	2/3	Lobar pneumonia	Meningo. C. B. inf.	129
R. L.	P. W. 38.	16	Alb. and casts	7800	Neg.	....	2/4	Bronchopneumonia. Sup. bronchiolitis.	Meningo. A. B. inf.	130

* Sup. Bronch. = Suppurative Bronchiolitis. † Predominating organisms only are recorded under lung cultures.

fever, headache, cough, pain in the chest and general malaise. The skin was pale blue. The patient looked very toxic; the chest examination showed variable areas of dullness and numerous moist râles, quite generally distributed. A few cases showed distinct lobar consolidation. The temperature was 103°-106°, pulse 85-100, respiration 25-30. The urine showed albumin and casts, usually early in the disease. The blood findings were negative blood cultures and a normal or reduced white cell count. The sputum was at first thin and watery, but rapidly changed to a creamy white which, on microscopic examination, showed numerous pus cells and gram-negative diplococci. Severe cases grew rapidly worse; the cyanosis increased; the lung consolidation extended and the patient died within a few days. Among those who survived the first week, there appeared to be a marked tendency toward suppurative bronchiolitis and protracted recovery. As the areas of consolidation diminished, the bronchioles filled with thick muco-purulent secretions; marked emphysema frequently developed; such patients showed marked evidence of dysphonia. At this stage, there was usually a marked rise in the white cell count. Later in the disease, the meningococci in the sputum became considerably reduced and other organisms correspondingly more prominent.*

AUTOPSY FINDINGS IN MENINGOCOCCUS PNEUMONIA

The lung findings at autopsy were usually of the broncho-pneumonic type with numerous points in the consolidated areas that exuded purulent fluid on pressure. Six cases were distinctly lobar in distribution. On section, the consolidated areas showed a peculiar pale violet color that appeared almost characteristic of meningococcus pneumonia. The pleura was generally quite free from exudate and the pleural surfaces were generally dry. Fibrinous pericarditis was present with very little increase of pericardial fluid. Endocarditis occurred in over half of the cases. Cloudy swelling of the liver, acute splenic tumor and parenchymatous nephritis were constant. The abdominal recti muscles were found ruptured in five cases. The upper bronchi were generally inflamed and covered with creamy pus. Meningococci were always found to be most numerous in the purulent exudate from the bronchioles. Cultures from the pericardium and pleural cavities never showed meningococci. One patient developed an empyema from which a pure culture of streptococcus hemolyticus was recovered from the fluid; yet, the lung cultures showed great predominence of meningococci. Meningococci were never recovered from any of the accessory sinuses. *B. influenzæ* was recovered from the sphenoidal cells in pure cultures in four cases.

Table III shows the laboratory findings in the 23 fatal cases of meningococcus pneumonia (without cerebrospinal involvement).

That virulent meningococci were prevalent among the troops in Camp Coetquidon is shown by the fact that from September 1, 1918, to February 15, 1919, 22 cases of cerebrospinal meningitis developed with 13 deaths. The meningococci isolated were of similar type to those found in the lung tissue of the meningococcus pneumonia cases.

BACTERIOLOGICAL FINDINGS IN CASES OF MENINGOCOCCUS MENINGITIS

Of the 22 cases of meningococcus meningitis that occurred during this period, blood cultures were taken in 16 with three positive results. The positive results were all obtained from early cases; and no positive blood cultures were obtained after the first day of the disease. In one case the spinal fluid was clear at the time the positive blood culture was secured. In this culture, the meningococci numbered 2 per c. c., yet on the following day, when the spinal fluid showed a high-cell count and numerous meningococci, a second blood culture gave negative results.

Spinal fluid cultures all gave positive results at the time the first turbid fluid was secured. In nearly all cases, however, cultures failed to grow meningococci after the third or fourth day, even though the white cell count remained high for much longer periods.

Panophthalmitis developed in one case and meningococci were recovered from the eye after enucleation. Among the 13 cases that came to autopsy, meningococci were recovered from the ventricles or base of the brain in six and from the pericardium in two cases. There was distinct evidence of pneumonic involvement in ten cases, from which cultures from section of the involved lung showed meningococci* in six cases (four times alone and twice associated with *B. influenzæ*). The other four cases gave lung cultures of streptococcus hemolyticus, pneumococcus Type IV, pneumobacilli and staphylococcus aureus, respectively. Eighteen meningococcus cultures, secured from cases of cerebrospinal meningitis that were typed, proved to be Type B Pasteur.

Meningococcus Carriers.—During the progress of the epidemic, efforts were made to segregate the carriers of meningococci in the various organizations that were showing the greatest number of cases. The carrier rate varied greatly at different times and in different barracks at the same time, the general rate being much higher early in the epidemic than later. Some of these carrier surveys were made on groups of men too small to furnish reliable figures. But four surveys were made at different times on fairly large numbers of men as follows:

Time	Number of men cultured	Carriers found	Percentage
Sept. 20/18	1160	198	16
Oct. 20/18	2286	280	12
Dec. 6/18	1254	110	8
Feb. 10/18	1196	60	5

These figures are of interest because they show an unusually high meningococcus carrier rate while the epidemiological waves were at their highest. The greatest number of carriers generally appeared to be present in the barracks where

* Thanks are due Captain W. M. Rhett, M. C., and the Medical Staff of Camp Hospital No. 15 for much of the clinical data.

respiratory affections were most numerous. Carriers isolated were recultured twice each week. Very few of them continued to be carriers over prolonged periods and no so-called "permanent carriers" were found during the investigation.

DISCUSSION

It will be observed that in this series meningococci rank third among the bacteria found most frequently in the lungs at autopsy in cases of pneumonia, being exceeded only by pneumococci and B. influenzæ. However, if cerebrospinal meningitis cases be included, the meningococcus becomes the most frequently found organism in the lesions causing death during the epidemic. Out of 50 strains of meningococci typed from carriers selected at random 44 were Type B, four Type C, and two Type A Pasteur. Eighteen cases of cerebrospinal meningitis were caused by Type B meningococcus. Nineteen autopsy cultures from cases of meningococcus pneumonia showed 14 Type B, three Type A, and two Type C Pasteur. It is, therefore, evident that the prevailing type of meningococcus during this period was Type B Pasteur and was responsible for all of the meningitis cases as well as the larger proportion of meningococcus pneumonia cases. The frequent association of meningococci with *B. influenzæ* is of special interest. On artificial media, the two organisms grow together exceedingly well. The possibility of a symbiotic pathogenicity of these organisms is worthy of further study.

CONCLUSIONS

1. That meningococci were an important factor in the causation of post-influential pneumonia at Camp Coetquidon, from September 1, 1918, to February 15, 1919.

2. That the meningococci present in the lungs in cases of meningococcus pneumonia were essentially the same type of organisms as those found in the spinal fluids in cases of epidemic cerebrospinal meningitis.

3. That cases of meningococcus pneumonia may arise from contact with cases of meningococcus cerebrospinal meningitis and *vice versa*.

4. The meningococci may produce either bronchopneumonia or lobar pneumonia.

TECHNIQUE OF BACTERIOLOGICAL EXAMINATIONS

Blood Cultures.—Routine blood cultures were made on all cases of pneumonia. The medium used for such routine cultures was prepared as follows: Peptone, 10.0; salt, 5.0; glucose, 5.0; beef infusion, 1000.0. This was titrated to +0.4 acidity to phenolphthalein; tubed in large test-tubes and sterilized in an autoclave at 30 pounds for 15 minutes.

Blood for culture was drawn from one of the arm veins with a sterile 10 c. c. syringe. This amount of blood was then injected into 40 c. c. of medium after which the mixture was incubated for 72 hours, unless growth occurred earlier. This method gave good results with pneumococci, streptococci and *B. influenzæ*, but trial cultures with meningococci were unsatisfactory.

Blood cultures for meningococci were made on a medium prepared by adding 2 per cent agar to the above-mentioned bouillon and sterilizing as before. This medium was melted and cooled to 45° C. The amount of blood drawn for each culture was 10 c. c. as before. This was mixed with 30 c. c. of the melted medium and poured into sterile petri dishes, allowed to set and placed inverted in the incubator for 72 hours unless growth occurred earlier.

Cultures of spinal fluids and sputa were made on a petri dish medium prepared by adding 2 per cent of fresh sterile human blood (kept fluid by citration) to the previously mentioned agar medium, after melting and cooling to 50° C. This was poured into sterile petri dishes and allowed to set. Spinal fluids were collected in sterile test-tubes and centrifuged. Preliminary examinations were made by microscopic examination of smears stained by Gram's method. Cultures were made by smearing the centrifuged sediment over the surface of the blood agar plates and incubating over night.

Sputa were obtained by having the patient cough and collecting the material directly into a sterile petri dish, from which direct smears were stained by Gram's method and examined. Cultures from sputum were made by selecting representative portions, smearing on blood-agar plates and incubating over night.

Agglutination Tests.—The sera used for preliminary agglutinations were Lederle's and the Rockefeller polyvalent antimeningococcus serum. The sera used for type determinations were Pasteur Institute Types A, B and C. All routine agglutinations were read macroscopically. The standard dilution was 1:50. Colonies resembling meningococci were smeared and stained by Gram's method and if found to be gram-negative diplococci, agglutination tests were made with standard sera. Subcultures, whenever necessary, were made by subculturing suspicious colonies either on Loeffler serum tubes or on blood-agar plates. The suspected colonies were emulsified with normal saline and this emulsion was mixed in equal proportion with polyvalent antimeningococcus serum diluted 1:25 with normal saline. A control tube containing normal saline instead of the antimeningococcus serum was set up in each case. In the early part of our work, control tubes made with normal horse serum were also set up. Later, these were discarded. The tubes containing the above mixtures were incubated for four hours at 37° C.

Positive cases were typed by substituting Pasteur Institute antimeningococcus sera A, B and C for polyvalent serum, using the same dilutions, controls and temperature of incubation as used for the preliminary agglutinations.

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MENINGOCOCCUS PNEUMONIA. II

THE EPIDEMIOLOGY OF POST-INFLUENZAL PNEUMONIA IN WHICH THE DIPLOCOCCUS INTRACELLULARIS MENINGITIDIS WAS ISOLATED. FROM OBSERVATIONS AT CAMP COETQUIDON, A. E. F., FRANCE

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Because of the occurrence of the cases of meningococcus pneumonia described in the preceding paper,[1] the Base Surgeon, Base Section No. 1, A. E. F., France, requested us to investigate the epidemiology of this condition.

This study includes all of the bacteriological examinations made at Camp Hospital No. 15, Camp Coetquidon, A. E. F., France, of contacts of meningococcus pneumonia and meningococcus meningitis cases during the latter part of September and the early part of October, 1918.

TABLE I

Situation of men examined	Number of men cultured	Number found positive, *i. e.* with meningococci in the rhinopharynx	Percentage of positive or carrier rate
Receiving wards	102	8	8 per cent.
Other hospital wards	456	32	7 per cent.
Discharged patients	596	85	14 per cent.
Contacts in barracks of 141st Field Artillery.	261	38	14 per cent.
Contacts in barracks of 142d Field Artillery.	86	8	9 per cent.
Contacts in barracks of 114th Trench Motor Battery.	66	13	20 per cent.
Contacts in barracks of 114th Ammunition Train.	6	0	0 per cent.
Total	1573	184*	12 per cent.

* The great majority of these carriers were of the temporary class. Of 152 meningococcus carriers cultured on the third day only 37 were found positive. Of 160 carriers at the next examination the sixth day only 15 were positive.

In order to determine whether the incidence of these cases was due to contact or infection in the hospital or in the barracks, cultures from the rhinopharynx were taken; (1) from every new patient at the time he was received into the hospital; (2) from all patients and attendants in the hospital; (3) from all patients at the time of their discharge from the hospital, and (4) from all contacts of recent cases of meningococcus meningitis in the barracks.

Until their cultures were reported negative, patients were held in strict quarantine in special wards of the hospital and discharged patients and contacts were held in "working quarantine" in the barracks, *i. e.*, they could drill and mingle with other troops out of doors but had to sleep in isolated barracks.

In all, 1573 different patients, attendants, and contacts were cultured. Cultures were repeated at three-day intervals on all men found harboring meningococci until two successive negative cultures were obtained.

In Table I the results of these cultures are summarized.

The numbers of cases of cerebro-spinal meningitis and pneumonia from which meningococci were recovered and the number of carriers found in each organization as a result of the surveys of patients in hospital and of contacts in barracks are indicated in Table II.

SUMMARY

From the necropsy and cultural investigations described in the preceding paper (Meningococcus Pneumonia I), there can be no doubt that the post-influenzal pneumonia in Camp Coetquidon was due in at least 23 cases to infection with the meningococcus. This conclusion is further substantiated by the epidemiology. The 140th, 141st and 142d brigades of field artillery came from Camp Beauregard (Louisiana) in which numerous cases of cerebrospinal meningitis (meningococcal) had occurred during the previous winter. Probably many men of these organizations still harbored meningococci in their throats. The epidemic of influenza with its coughing greatly increased the number of these meningococcus carriers.

[1] See p. 324 of this issue.

The cultural surveys of the hospital patients and barrack contacts (Table I) show an average meningococcus carrier rate of nearly 12 per cent. From the wide distribution, as shown

TABLE II

Organization	Number of meningococcus pneumonia cases	Number of meningococcus meningitis cases	Number of meningococcus carriers found while patients in hospital	Number of meningococcus carriers found in survey of barracks	Total number of meningococcus carriers found in each organization
131st Field Artillery.	5	0	3	No survey.	3
132d F. A.	1	0	3	No survey.	33
133d F. A.	2 (1 fatal).	0	5	No survey.	5
140th F. A.	3 (1 fatal).	0	12	No survey.	12
141st F. A.	5 (4 fatal).	4 (1 fatal).	34	38	72
142d F. A.	0	2 (2 fatal).	10	8	18
328th F. A.	1	0	1	No survey.	1
329th F. A.	1 (fatal).	0	14	No survey.	14
330th F. A.	0	1	2	No survey.	2
114th Ammunition Train.	1 (fatal).	1	29	0	29
310th A. T.	0	0	2	No survey.	2
114th Trench Mot. Bat.	1 (fatal).	1 (fatal).	0	13	13
61st Engineers.	2 (1 fatal).	0	3	No survey.	3
Casuals...	1 (fatal).	0	7	No survey.	7
Total...	23 (11 fatal).	9 (4 fatal).	125	59	184

in Table II, it was impossible to state definitely whether the infections occurred after the patients were received into the hospital or whether they had occurred in the barracks, but it it probable from the high carrier rates in both places that the influenza patients might well have acquired their secondary infection in either location.

It would seem evident, therefore, that the meningococcus was both endemically and epidemically distributed in the rhinopharynges of the troops in Camp Coetquidon and that this explained the relatively large number of cases of post-influenzal pneumonia in which meningococci were isolated.

TECHNIQUE USED IN EXAMINATION OF MENINGOCOCCUS PNEUMONIA AND MENINGITIS CONTACTS

Swabs were taken from the posterior rhinopharyngeal wall (curved wire swab "A" and tongue depressors "B" being used) and immediately inoculated on a small area of a hemolysed blood-agar plate. (Veal infusion agar + 0.2 per cent to phenolphthalein, or Ph. 7.8 was used; 3.0 c. c. of human, sheep, or rabbit's blood, hemolysed with 10 c. c. of sterile distilled water, were added to each 100 c. c. of melted agar at 50° C., immediately before pouring plates). This inoculation was then spread over the whole surface of the plate by means of a sterile wire spreader (C), (either immediately or on return to the hospital laboratory) and the plates incubated for from 18 to 24 hours at 37° C. They were then examined for suspicious colonies with the naked eye or a 10-power hand-lens. Meningococcus colonies are round, transparent, light gray and not umbilicated. Suspicious colonies were fished to hemolysed blood agar or Loeffler coagulated serum slants and incubated over night. A gram-stained smear was then made from each subculture.

Those containing gram-negative cocci were emulsified in normal saline as follows:

1. Saline was added to the cultures with a pipette according to the amount of growth (*i. e.*, 2 to 3 c. c.).

2. The growth was carefully washed off by means of a dropping pipette (*i. e.*, one fitted with a nipple) and transferred to a second tube.

3. Saline solution was added to these suspensions so that all were distinctly and equally cloudy.

4. The emulsions were agglutinated with meningococcus serum, the following technique being used: Five drops, or

TABLE III

To the 1st tube.	5 drops or 0.25 c. c. of the bacterial suspension.	5 drops or 0.25 c. c. Rockefeller Institute or N. Y. Board of health polyvalent meningococcus serum.	Previously diluted to 1:25.	Making the final dilution 1:50.
To the 2d tube.	" "	5 drops or 0.25 c. c. Pasteur type A meningococcus serum.	"	"
To the 3d tube.	" "	5 drops or 0.25 c. c. Pasteur type B meningococcus serum.	"	"
To the 4th tube.	" "	5 drops or 0.25 c. c. Pasteur type C meningococcus serum.	"	"
To the 5th tube.	" "	5 drops or 0.25 c. c. Pasteur type D meningococcus serum.	"	"
To the 6th tube.	" "	5 drops or 0.25 c. c. normal saline.	Control.	

0.25 c. c. of the bacterial suspension and the same quantity of various meningococcal type sera were placed in each of five agglutination tubes as indicated in Table III.

The five tubes were then incubated at 37° C. preferably for two hours in a water-bath or three hours in a dry incubator.

Lt. Col. M. H. Gordon's meningococcus type sera, I, II, III and IV or the Rockefeller Institute normal, paranormal, and intermediate type sera may be substituted for the Pasteur Institute type sera A, B, C and D.

When many cultures are to be agglutinated it will be found more economical of time and material if only tubes 1 and 6

are used. If after two hours there is positive agglutination with a polyvalent serum, the original suspension may then be typed by agglutinating in tubes 2, 3, 4 and 5.

The men whose cultures gave positive agglutination with a polyvalent serum were reported immediately as carriers, placed under hospital or working quarantine (*vide supra*), and re-

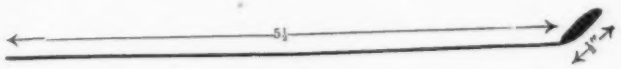

FIG. 1.

examined at three-day intervals until two successive negative cultures were obtained.

APPARATUS

A. Ordinary hay wire was cut into six-inch lengths and the distal half-inch was bent at an angle of 135° with the shaft. Non-absorbent cotton was twisted around this distal curved end. These were sterilized by dry heat in lots of 25 in metal containers or glass jars, the tops of which were covered with paper. In our opinion, this swab is more convenient than the West tube swab not only on account of its greater simplicity of preparation but from the fact that any one with a little practice

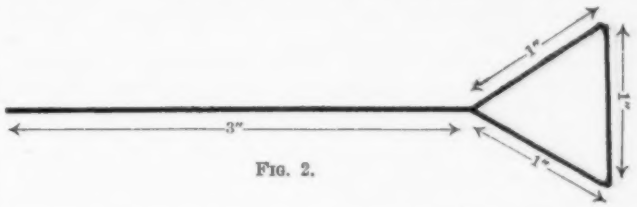

FIG. 2.

can swab the posterior rhinopharyngeal wall of an adult without touching the mouth structures.

B. It was found that requiring each man to use his own mess spoon as a tongue depressor was often convenient.

C. To make spreaders six-inch lengths of hay wire were bent as shown in the diagram. These were sterilized by dry heat in lots of 25 in metal or glass containers.

THE OCCURRENCE OF THE PFEIFFER BACILLUS IN MEASLES

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Certain similarities between measles and influenza were especially noticeable when these diseases were prevalent during the early part of last autumn. With the onset of influenza at Camp Devens, Mass., the symptoms in the earlier cases were distinctly less fulminating than at the height of the epidemic. The possibility of measles in the pre-eruptive stage required consideration in establishing the diagnosis of the first group of influenza patients admitted to the base hospital. The acute onset of conjunctival and respiratory symptoms was accompanied by a pronounced leucopœnia. There are other noteworthy similarities between the two diseases. Both are highly contagious and are frequently complicated by pneumonia in its various forms. The occurrence of the Pfeiffer bacillus in a high proportion of patients has been reported in measles as well as influenza. As we were engaged at this time in the study of measles, it seemed advisable to investigate the occurrence of Pfeiffer and Pfeiffer-like organisms in measles patients.

Summary of Literature.—The literature contains fairly numerous reports of the occurrence of *B. influenzæ* of Pfeiffer in cases of measles. Unfortunately a considerable number of the reports fail to furnish the information upon which the identification of the bacillus was based. In some, reliance seems to have been placed upon microscopic examination of smears stained by Gram's method; in other instances typical cultures were obtained upon blood-agar but no evidence was furnished to show that hæmoglobin was essential for successful cultivation. Such results are open to doubt since other organisms may simulate closely the growth of *B. influenzæ* on blood-agar. In the following review, as a rule, those papers will be omitted in which pneumonia and a Pfeiffer bacillus infection are reported as remote sequelæ of measles.

The association of measles and the influenza bacillus was first mentioned by Paltauf[1] in 1899. He reports the occurrence of *B. influenzæ* in two cases of bronchopneumonia following measles; the details concerning the means of identification of the bacillus are not stated.

Giarrè and Picchi[2] were the first investigators to report the occurrence of Pfeiffer-like organisms in active cases of measles, pure cultures being recovered readily from the conjunctival and bronchial secretions. These authors raise the question of a possible specific etiologic rôle of these organisms in measles.

Albrecht and Ghon[3] in 1901 describe one case of bronchopneumonia developing three weeks after an attack of measles. At autopsy the influenza bacillus was isolated from the lung.

In the autopsy of 23 cases of measles Jehle[4] reports the occurrence of the Pfeiffer bacillus in the heart's blood in 15 and in the lungs in 18 cases. This author regards the influenza bacillus as a common secondary invader in the acute exanthemata.

Süsswein[5] in 1901 studied measles patients clinically and at autopsy; he recovered the Pfeiffer bacillus in 10 of 21 cases. The positive cases were considered to be mixed infections of measles and influenza.

Liebscher[6] in autopsies of 11 cases of measles, recovered the Pfeiffer bacillus in 3 cases from the lungs; his work is of especial interest in that two of three cases showed a pneumonia with the influenza bacillus present in pure culture.

Klienberger[7] reports cultivation of "hæmophilic" bacilli from the conjunctivæ in 3 of 9 patients of measles during life, and in one of two cases at autopsy a pure culture of "hæmophilic" bacilli was obtained from the heart's blood.

Davis[8] examined uncomplicated cases of measles during the stage of eruption. Cultures were taken from the sputum or from throat swabs, a single examination being made in each case Organisms indistinguishable from the Pfeiffer bacillus were isolated in 13 of 22 cases.

Pacchioni and Franchioni[9] obtained Pfeiffer-like organisms readily from the mucous membrane of measles cases. Careful blood cultures in 25 patients were negative for this organism in all except one case; one positive culture was obtained shortly before death from a case complicated by bronchopneumonia. These authors give serious consideration to the possibility that measles may be caused by a Pfeiffer-like organism.

Reiss and Gins[10] report the cultivation of the influenza bacillus from the blood 17 days before death from a case of postmeasles bronchopneumonia.

Of especial interest are those reports which record the presence of the Pfeiffer bacillus in the uncomplicated cases of measles during life. The bacillus has been found, as a rule, only in the mucous membrane of the conjunctivæ and in the respiratory tract. Only rarely has it been recovered by blood culture and then only in cases complicated by pneumonia. It is noteworthy that Liebscher in 1903 reported the occurrence of the Pfeiffer bacillus in pure culture in the lungs in two fatal cases of pneumonia following measles.

EXPERIMENTAL WORK

The following examinations were conducted chiefly at Camp Devens, Mass., and were commenced immediately after the decline in the epidemic of influenza in September and October, 1918. The latter began on September 8, reached its maximum 8 days later and declined very rapidly during the next week. In the remainder of September and October a few cases were admitted practically every day; by the first of November the admissions for influenza had virtually ceased. Small foci of the infection probably remained in camp throughout the winter. A full account of the epidemic was reported by Woolley.[11] The measles patients were examined with the object of determining the relative frequency of Pfeiffer or Pfeiffer-like organisms and the period for which these organisms persisted after the subsidence of the symptoms of measles. Numerous cultures of hæmoglobin requiring organisms were isolated; these strains were, superficially at least, identical with *B. influenzæ* of Pfeiffer.

Technique.—The cases of measles were examined during the stage of eruption, the first examination being made as a rule within from 24 to 36 hours after the appearance of the rash; subsequent examinations were made at intervals of one to two weeks. Only a single examination was made during the eruptive stage in the cases which were negative. All of the patients showed the usual respiratory symptoms but only two of the cases were complicated with a definite pneumonia. As a routine the sputum of patients was examined, material being secured, as far as possible, which had been coughed up from the trachea or bronchi. In the hope of eliminating some of the commoner saprophytes, the sputum was injected in quantities of 0.25 to 0.5 c. c. intraperitoneally into white mice according to the technique recommended by the Rockefeller Hospital for the isolation of the pneumococcus. The mice were sacrificed when they were markedly ill, usually after about 10 to 12 hours, spontaneous deaths being for the most part avoided. Cultures were made on blood-agar plates. Throughout this work human blood-agar has been employed—10 per cent blood in meat infusion agar with a reaction to phenolphthalein equivalent to 0.1 per cent of normal acid. These plates were inoculated from the peritoneal exudate, from the heart's blood and from any early pneumonic or hæmorrhagic patches in the lung.

The growth from the heart's blood and from the lung often showed an almost pure culture of Pfeiffer-like organisms, *i. e.*, a small nonmotile Gram-negative bacillus (about 0.2 by 0.5 micron) without capsule or spore formation, growing aërobically on human blood-agar as minute discrete colonies without producing hæmolysis. The need for hæmoglobin was tested by substituting

TABLE I

INCIDENCE OF PFEIFFER-LIKE BACILLI IN SPUTUM OF MEASLES PATIENTS

Case number	Date of first examination	Period of observation			
		During eruptive stage	10 to 14 days after eruption	20 to 25 days after eruption	40 days after eruption
1	Nov. 12	Positive	Negative	.....	.....
2	" 12	"	Positive	.....	Negative
3	" 12	"	Negative	Negative	"
4	" 12	"	"	"	.....
5	" 12	"	"	.....	.....
6	" 12	"	"	Negative	.....
7	" 26	"	Positive	"	.....
8	" 26	"	"	Positive	.....
9	" 26	Negative	Negative	"	.....
10	" 26	Positive	"	"	Negative
11	" 26	Negative	"	Negative	.....
12	" 26	Positive	"	"	.....
13	" 28	Negative	"	"	.....
14	" 28	Positive	"	"	.....
15	" 28	.....	"	Positive	Negative
16	" 30	Negative	"	"	.....
17	" 30	Positive	Positive	Positive	Negative
18	" 30	"	"	Negative	.....
19	" 30	"	Negative	"	.....
20	" 30	"	"	"	.....
21	" 30	"	"	"	.....
22	Dec. 2	"	Positive	"	.....
23	" 2	Negative	Negative	"	.....
24	" 4	Positive	"	"	.....
25	" 4	"	"	"	.....
Total		Positive 19, or 79%	Positive 6, or 24%	Positive 6, or 26%	Positive none

ascitic fluid for blood in the culture medium—10 per cent of unheated ascitic fluid, free from bile pigments, with a specific gravity of 1.018. Blood serum was not employed on account of extremely minute amounts of hæmoglobin which may sometimes suffice for the growth of the Pfeiffer bacillus. In testing the hæmoglobin requirements of a given strain, blood and ascitic media were prepared from a common stock of agar. Heated blood-agar (chocolate-agar) was occasionally employed. Agar of the same composition as used for the fresh blood medium was mixed with human blood (10 per cent). The mixture was maintained at a temperature of about 95° C. for approximately ten minutes.

Strains of a Pfeiffer-like organism were recovered from five of the first six cases of measles that were examined. Seven normal men on duty at the base hospital were tested in the same way. Two of these men had had influenza 6 weeks before. No organisms resembling the Pfeiffer bacillus were found; these tests were repeated three and seven days later with nega-

tive results. The following table gives the incidence of the Pfeiffer-like organisms found in 25 additional cases of measles.

The cases in Table I are given in chronological order to show that the positive results occurring on the first examination were distributed rather equally throughout the series.

Distribution of the Pfeiffer-like Organisms.—Examinations were made for determining the extent to which the hæmoglobin requiring organisms are distributed throughout the body in cases of measles. Cultures for this purpose were made during the first day of the rash from the conjunctivæ, from morbillous lesions of the skin, from the blood and in two cases from lymphatic glands.

Conjunctivæ.—Inoculations from the inflamed conjunctivæ were made with a cotton swab on blood-agar plates. The secretions showed comparatively few bacteria of any kind. Fourteen cases were examined and Pfeiffer-like cultures were recovered from six; in two of these cases the organisms were present in relatively large numbers in almost pure culture.

Skin Lesions.—Small pieces of skin showing morbillous lesions were excised and smeared on blood-agar. Eight cases were examined but no hæmoglobin-requiring organisms were recovered.

Blood Cultures.—In view of the almost constantly negative results of blood cultures for the Pfeiffer bacillus in influenza, comparatively few examinations were made in the measles cases. Meat infusion broth containing a little laked human blood was inoculated with the patient's blood during the first 12 to 24 hours of the rash, from 1 to 5 c. c. of patient's blood with 75 to 100 c. c. of broth being used. Large Erlenmeyer flasks were employed as containers for the broth in order to give a large surface area. After incubation for 24 to 48 hours, subinoculations were made from these flasks on ordinary blood-agar and on heated blood-agar plates. The results in seven cases were negative. Six of these were uncomplicated. One patient developed early pneumonic lesions; the cultures were continued during the 2d, 3d and 4th day of the rash, but only a non-hæmolytic streptococcus was recovered from the blood.

Lymphatic Glands.—In a rather large number of the infectious diseases the causative organisms can be recovered from the lymphatic glands more readily than from the blood stream. In measles there is at least a theoretical possibility that the virus is disseminated by way of the lymphatic system. In two cases, a superficial gland was excised from the inguinal region as soon as the rash had advanced to this area. Smears from the gland were made on blood-agar and on heated blood-agar plates. The results in one case were negative; in the other, 5 or 6 colonies in all were obtained on the heated blood-agar, no growth developing on the unheated media. Sub-cultures were obtained either on heated or unheated blood media but only with difficulty; no growth was obtained on ascitic agar. Even on heated blood-agar the colonies were always much smaller than the typical Pfeiffer colonies; sometimes growth could be definitely demonstrated only by the microscopical examination of a stained smear. In its morphology and staining reactions, this organism corresponded to the Pfeiffer bacillus except that, even in very young cultures on unheated blood-agar, numerous forms were present which were from two to four times the length of the typical Pfeiffer organism. After four sub-inoculations the strain died out.

COMPARISON OF PFEIFFER STRAINS FROM CASES OF MEASLES AND OF INFLUENZA

These strains of organisms from the conjunctivæ and the sputum were propagated on human blood-agar, transfers being made at intervals of four or five days. They were compared with similar strains of the Pfeiffer organism isolated at autopsy from the lungs of influenza cases occurring during the epidemic. Morphologically the strains from the two diseases were almost identical. The cultures from the measles cases showed little tendency to develop bizarre forms even on heated blood-agar and then never in extreme degree. Davis[8] has reported the isolation of a strain of Pfeiffer bacillus possessing feeble hæmolytic properties. No hæmolysis was produced by our strains on human blood-agar or broth media made of 5 per cent of blood in meat infusion broth just acid to phenolphthalein (0.1 per cent normal acid). Only very scanty growth occurred in the unheated blood broth medium. Fairly abundant growth was secured in liquid media by partially laking the blood with two volumes of water. Preliminary observations were carried out on the spectroscopic changes occurring in cultures. Uninoculated control flasks of media showed typical absorption bands of oxyhæmoglobin. With whole blood slight growth occurred without any apparent change in the absorption bands. With laked blood after 48 hours the cultures showed bands characteristic of methæmoglobin; after 72 hours, in acidified samples a broad band also appeared in the green. Cultures from measles cases produced changes more readily than those obtained from the influenza patients, but these differences might readily be explained by a difference in the rate of growth.

The principal object of the comparison of the measles and influenza strains consisted in an attempt to find some fundamental biological differences between the organisms obtained from these two sources. Observations were conducted on the reactions of agglutination and complement fixation, on the resistance of the organisms to freezing and drying and on the effect of bile and of sodium hydroxide. A comparison was made of the local reaction of the two strains upon intracutaneous inoculation of rabbits; strains from measles cases were also inoculated into monkeys and into some human volunteers.

Immunity Reactions.—Both the agglutination test and the complement fixations failed to show any constant differences between the two strains of organisms; some of the measles strains reacted with the serum of measles and influenza convalescents and also with normal individuals. In some instances the serum of a measles convalescent fixed complement with strains of organisms obtained from either measles or influenza patients. No evidence was obtained which possessed any significance regarding the etiology or diagnosis of measles. In some experiments on phagocytosis, Pacchioni and Franchioni[9] re-

port that the serum of measles patients possesses a high opsonic index for Pfeiffer-like organisms.

For the agglutinations, a moderately heavy suspension of bacteria was prepared in physiological saline from a 48-hour growth on blood-agar. This suspension was mixed with an equal volume of serum in dilutions varying from 1-10 to 1-100. The mixtures were incubated at 37°C. for from 2 to 24 hours and at 55°C. for 12 hours. The majority of all the results were negative; only a comparatively few preparations showed well defined clumping.

The complement fixations were carried out with a sheep cell rabbit serum hæmolytic system, two units of complement being used. The antigen was prepared by digesting a saline suspension of the bacteria at 37°C. for 5 to 6 days. After centrifugalization, the opalescent supernatant fluid was titrated for its antigenic power; one-half of the maximum quantity permitting complete hæmolysis was employed in the tests. The serum to be tested was inactivated at 55°C. and 0.1 c. c. was employed; the total volume of the final preparation was 2 c. c. One series of antigens was prepared by alcoholic extraction of a saline suspension of bacteria that had been allowed to autolyze for one week at 37°C. Since these immunity reactions were essentially negative the results are not recorded in detail.

The results of the agglutination tests conducted by Wollstein [23] indicate that the Pfeiffer-like bacilli can be subdivided into smaller groups. Such evidence, unless very striking differences are obtained, does not constitute proof of distinct species. Accordingly, we did not carry the work on immunity reactions any farther. Some of the more general biological characteristics of these two strains of Pfeiffer organisms have been investigated in the search for evidence that might indicate the existence of distinct species in the hæmoglobinophilic group.

TABLE II

SHOWING EFFECT OF FREEZING ON PFEIFFER-LIKE ORGANISMS

Serial number	Growth on blood-agar after		
	5 freezings	10 freezings	15 freezings
STRAINS FROM MEASLES CASES			
2	Heavy	Heavy	Heavy
4	"	.....	None
7	"	Slight	"
10	"	Heavy	Heavy
12	"	"	"
26	"	Slight	"
27	"	Heavy	"
28	"	"	"
STRAINS FROM INFLUENZA CASES			
A	Heavy	Slight	Heavy
B	"	"	None
C	"	Heavy	Heavy
D	"	Slight	None
E	"	.....	Heavy
F	"	Heavy	None
G	"	None	"
H	"	Heavy	"

Effect of Freezing.—Many of the Pfeiffer cultures were found to be susceptible to even moderately low temperatures whereas others were resistant. The measles and the influenza strains did not fall into distinct groups. However, six of eight cultures from measles cases in contrast to three of eight cultures from influenza patients were resistant to freezing.

Technique.—Emulsions of the Pfeiffer bacillus in physiological salt solution were rapidly frozen and thawed 15 times, a freezing mixture of ice and salt at about — 10° to — 12° C. being used. After each 5 freezings, cultures were made on human blood-agar plates. Table II gives a typical result.

Several repetitions of this experiment on these same cultures showed that the behavior of the individual strains was almost constant. Some of the strains were not only unable to withstand alternate freezing and thawing but were destroyed by continuous freezing at about — 10° C. maintained for from two to three hours.

Effect of Drying.—These cultures of the Pfeiffer bacillus succumbed rather readily to drying for a short period. Under the conditions of the experiment, the measles strains survived a little longer than those from influenza patients but the differences were not marked.

TABLE III

SHOWING EFFECT OF DRYING ON PFEIFFER-LIKE ORGANISMS

Serial number	Growth on blood-agar	
	After 4 minutes	After 8 minutes
STRAINS FROM MEASLES CASES		
2	None	None
4	"	"
7	Alive	Alive
10	"	"
12	"	"
26	None	None
27	Alive	Alive
29	"	"
STRAINS FROM INFLUENZA CASES		
A	None	.....
B	Alive	Alive
C	None	None
D	Alive	Alive
E	None	None
F	"	.....
G	"	None

Technique.—A coarse coil of platinum wire was dipped in a saline suspension of young cultures of the organism to be tested. The moistened coil of wire, free from any gross droplets of the emulsion, was suspended in a dessicator at room temperature for periods of 4, 8 and 12 minutes. Cultures were then made on blood-agar. A characteristic result is given in Table III, these results being constant on two repetitions except in the case of one culture (4) which was dead in one and alive in another of the two succeeding tests.

Action of Bile.—In contrast to their resistance to freezing and drying, the measles strains seemed more susceptible to the action of bile than the cultures obtained from influenza cases.

Technique.—Saline suspensions of 48-hour cultures of the Pfeiffer organisms were mixed with an equal volume of varying dilutions of bile. The mixture was incubated for 1 hour at 37°C. in a water-bath. Occasionally there was some macroscopic evidence of clearing, but in order to obtain definite results, cultures were made upon blood-agar. A similar technique was used in the later

experiments in which bile salt and sodium hydroxide were substituted for bile. The dilutions given in the tables represent the final strength after mixture with the bacterial emulsion.

The action of bile is illustrated in Table IV.

The difference in behavior of the two strains towards bile appears at first to be very striking; moreover, the individual

TABLE IV

SHOWING GROWTH ON BLOOD-AGAR AFTER EXPOSURE TO BILE

Serial number	Final dilution of bile			
	1:5	1:10	1:20	1:40
STRAINS FROM MEASLES CASES				
1	None	None	None	Slight
2	"	"	Slight	"
4	"	"	"	Heavy
7	"	"	"	"
10	"	"	"	"
12	"	"	None	Slight
26	"	"	Slight	Heavy
27	"	"	"	"
29	"	"	"	Slight
STRAINS FROM INFLUENZA CASES				
B	Heavy	Slight	Heavy	Heavy
C	"	Heavy	"	"
D	Slight	Slight	Slight	Slight
E	None	None	None	None
F	Heavy	Heavy	Heavy	Heavy
G	"	Slight	Slight	"
H	Slight	Heavy	Heavy	"

cultures in four repetitions of this test behaved constantly in this manner. However, the total number of cultures tested is small and even in moderate dilutions of 1: 40 the difference disappears altogether.

Action of Sodium Hydroxide.—The effect of sodium hydroxide upon the cultures was rather variable, but the results in general were the reverse of those obtained with bile. The following data in Table V are characteristic.

TABLE V

SHOWING GROWTH ON BLOOD-AGAR AFTER EXPOSURE TO SODIUM HYDROXIDE

Serial number	Final dilution of sodium hydroxide				
	N/5	N/10	N/20	N/50	N/100
STRAINS FROM MEASLES CASES					
1	None	None	Slight	Heavy	Heavy
2	"	.....	"	Slight	Slight
4	"	None	"	Heavy	Heavy
7	"	"	"	"	"
10	"	"	"	"	"
12	"	"	"	Slight	Slight
26	"	"	None	None	"
27	"	"	Slight	Heavy	Heavy
29	"	"	None	"	"
STRAINS FROM INFLUENZA CASES					
B	None	None	None	Slight	Heavy
C	"	"	"	None	Slight
D	"	"	"	Slight	Heavy
E	"	"	"	"	Slight
F	"	"	"	None	"
G	"	"	Slight	Heavy	Heavy
H	"	"	None	Slight	Slight

From Table V it will be seen that there is only a small range of dilutions within which it is possible to demonstrate differences with sodium hydroxide; moreover, with some strains of organisms it is very difficult to obtain constant results under apparently identical conditions.

Action of Bile Salts.—Whole bile contains not only the biliary salts but an appreciable amount of alkali; since bile and the hydroxide act in a somewhat different manner on these Pfeiffer strains, it seemed desirable to try the action of bile salts alone. As in the case of other manifestations of the action of bile, the effect of the purified salt was less striking than the action of the whole product. Sodium taurocholate in 2½ per cent concentration killed all cultures tested; at 1 per cent the majority of the cultures were killed, whereas with one-half per cent about half of the measles and also half of the influenza strains were killed; at one-tenth per cent, all but 3 of 16 strains lived. Certain strains of the Pfeiffer bacillus are therefore approximately as susceptible as the pneumococcus to the purified bile salt.

Effects of Inoculation.—These cultures exhibited very little virulence upon inoculation into the smaller laboratory animals. Four cultures of measles strains and three of the influenza strains were tested upon rabbits, guinea-pigs, and mice by intraperitoneal inoculation. The rabbits and guinea-pigs showed no symptoms; occasionally the mice were killed in from two to three days by the injection of the growth from one to two blood-agar slants. A series of rabbits was inoculated intracutaneously with the two strains of organisms. Seven cultures from measles patients were tested; in all cases well marked œdema and inflammation developed during the first 24 hours, these symptoms persisting for from two to four days. Of seven cultures from influenza cases, tested in a similar manner, five showed virtually no reaction and two behaved precisely like the cultures from measles cases. The influenza cultures had been isolated approximately two months earlier than those obtained from measles patients; the two influenza strains producing inflammation were obtained earlier in the epidemic than the five causing little or no reaction.

Two adult monkeys (*Macacus rhesus*) were inoculated with a mixture of eight strains obtained from the sputum and conjunctivæ of measles cases. These cultures had been isolated for periods varying from one to three weeks. The growth from 48-hour cultures on blood-agar plates was suspended in salt solution. This emulsion was thoroughly rubbed over the mucous membranes of the eyes, nose, mouth and throat. In one monkey the mucous membrane of the mouth was scarified slightly on the right side; on the left side 0.5 c. c. of the bacterial suspension was injected under the mucous membrane. The scarified mucous membrane healed promptly and no local lesion developed at the site of the inoculation under the mucous membrane. Each of these animals also received intraperitoneally 2 c. c. of a moderately heavy saline suspension of these cultures.

Daily temperatures and white counts were made upon both monkeys. One remained entirely normal; the other, after an incubation period of six days developed definite malaise as evidenced by the loss of strength and loss of appetite. This animal had been under close observation for four months prior to these injections and had remained entirely free from any symptoms. During the period of malaise a suggestive drop occurred in the leucocyte count, unaccompanied, however, by any febrile reaction. The symptoms disappeared before the normal white count was fully re-established. At frequent intervals after inoculation, cultures were made from the mucous membranes on blood-agar. Pfeiffer-like organisms were not recovered from either monkey. The temperature and counts in the monkey which became ill were as follows:

INOCULATION OF MONKEY WITH PFEIFFER-LIKE STRAINS OF ORGANISMS

Days after inoculation	Temperature	White count	Room temperature
..	101.8	13,750	66°
1	100.8	25,250	63.5°
2	101.6	13,600	67°
3	100.2	23,800	77°
4	101.4	15,300	78.8°
5	100.4	14,600	66°
6	101.2 [1]	10,000	68°
7	101.0 [2]	10,800	68°
8	101.6 [3]	10,600	68°
9	101.2 [4]	15,500	68.9°
10	102.6 [4]	9,700	66°
11	102.0	11,100	68.9°
12	101.8	15,400	68°
13	101.6	16,000	68°
14	103.2	13,300	68.9°
15	102.5	15,300	68.9°
16	101.6	16,200	68.9°
17	102.0	17,300	68°
18	101.2	15,700	67°
19	100.6	16,400	68.9°
20	100.4	17,400	66°

[1] Cyanosis, conjunctivitis and loss of appetite.
[2] Deep cyanosis, critically ill.
[3] Marked improvement, eyes clearing, appetite good.
[4] Apparently normal.

A few inoculations of human volunteers were undertaken with the Pfeiffer strains obtained from measles cases. A mixture of five strains was employed, three of which were obtained from the sputum and two from the conjunctivæ. These cultures had been under artificial cultivation approximately six to eight weeks. All were easily killed by bile and all were resistant to freezing.

For the protection of the individual, the precautionary measures were taken, which have already been outlined for the inoculation of volunteers with the virus of measles;* these precautions include observations for the exclusion of the Pfeiffer bacillus. As an additional safe-guard for the first inoculation, an individual was chosen who was immune to measles and who had had influenza in the recent epidemic. The mucous membrane of the eyes, nose and throat was inoculated with a saline emulsion of the five strains. During the next two weeks cultures were made at three-day intervals on blood-agar and mice were inoculated with sputum for the recovery of the Pfeiffer organism. The cultures were negative and the individual himself developed no symptoms.

Accordingly, a volunteer was inoculated who had had influenza recently but not measles. No symptoms appeared and the cultures failed to colonize. Two additional volunteers were inoculated, who as far as could be determined, had had neither measles nor influenza. Both of these subjects remained entirely free from symptoms; the white blood count and the temperature did not vary beyond the normal limits, and the cultural examinations failed to show any evidence of colonization of the inoculated organisms. Under natural conditions, the Pfeiffer bacillus can readily establish itself in the mucous membranes. Hence the failure to produce symptoms would have possessed much more significance if successful colonization of the inoculated cultures had been obtained. These negative results are of some interest in view of the discussion regarding the occurrence of *B. influenzæ* in normal throats; in some instances, at least, it does not persist when heavily inoculated into healthy individuals.

Davis* found the Pfeiffer organism in a high percentage of cases of pertussis. Under the more favorable circumstances of an interepidemic period, this investigator reports the production of respiratory symptoms with prolonged colonization of the bacillus in man.

DISCUSSION

A comparison of different strains of the Pfeiffer bacillus showed considerable variation, more especially as regards their resistance to freezing and to the destructive action of bile. The attempt to separate the cultures into two distinct groups becomes very difficult during an epidemic on account of the opportunity for mixed and for cross infection. It is to be expected, for example, that a group of cultures obtained from measles cases would contain some strains behaving like the majority of those obtained from influenza. Such variations were numerous, but the individual strains were not consistent in the series of tests that were employed. Thus, two of the measles strains were susceptible to freezing. In this respect they behaved like the influenza cultures; yet these same strains were resistant to drying and susceptible to the action of bile, conforming in these tests to the majority of the other cultures from measles. The complete record of these strains that were tested is shown in the following outline.

VARIATIONS IN PFEIFFER-LIKE ORGANISMS

Tests employed	Freezing	Drying	Bile 1–10	NaOH N/20	Intracutaneous inoculations
Serial number of measles strains resembling influenza cultures	4 7	2 4 26	None	26 29	17 29
Serial letter of influenza strains resembling measles cultures	A C E	B D	E	G	B I

Our information concerning the Pfeiffer bacillus is as yet extremely meagre; satisfactory methods have not yet been

* Bull. Johns Hopkins Hosp., 1919, XXX, 257.

devised for performing tests for pathogenicity or for fermentation and immunity reactions. At present this bacillus is identified by its size, the lack of motility, its decolorization by Gram's stain and its multiplication only in the presence of hæmoglobin.* The situation may be illustrated by comparison with the very broad typhoid-colon group of organisms. As regards *B. coli* and *B. typhosus* these organisms are virtually identical in their size, motility and staining characteristics. Without either fermentation tests or immunity reactions it would become a difficult problem to separate them into perfectly distinct species or even to determine the etiology of typhoid fever.

In the absence of definite information it seems unwise to attempt any final statements concerning *B. influenzæ* and its possible etiologic rôle in the respiratory diseases in which it is most commonly found. Certainly the general opinion at present is decidely against the acceptance of the bacillus as the specific etiologic agent of influenza. This opinion is held largely because of the frequent failure to find the organism in typical cases and because of its common occurrence in other respiratory diseases. As in pneumonia it is conceivable that various organisms might give rise to more or less definite symptoms of influenza; the Pfeiffer-like bacilli, moreover, are not more widely distributed in healthy individuals than is the pneumococcus. Some consider that *B. influenzæ* is purely a secondary invader in a variety of diseases initiated by other agents; when the bacillus is found in pure culture in lesions of the lungs, the natural explanation of a primary etiologic action is set aside and a hypothetical virus is postulated. However, there is good reason to believe that serious symptoms may result when an infection with the Pfeiffer bacillus has become established; its acceptance as a specific etiologic agent, however, seems to us to be contingent upon some division of these organisms such as the recognition of distinct species or of virulent and avirulent varieties.

SUMMARY

1. The examination of a group of measles cases occurring a few weeks after an epidemic of influenza showed the presence of an organism indistinguishable from the Pfeiffer bacillus in 25 of 31 cases.

2. This organism was obtained readily from the sputum and with little difficulty from the conjunctivæ. A highly parasitic, hæmoglobin-requiring organism was obtained in one of two cases from an excised inguinal gland. The Pfeiffer organism was not obtained from the blood stream nor from the excised skin lesions.

3. With the subsidence of the active symptoms of measles these micro-organisms disappeared rather rapidly in about three-fourths of the cases.

4. Cultures of the Pfeiffer organism from cases of measles failed to colonize when inoculated on the mucous membrane of four healthy volunteers; two of these individuals supposedly had not had either measles or influenza.

5. A comparison was made of the strains of the Pfeiffer bacillus isolated from measles and from influenza. The results showed considerable variation in the behavior of the individual strains. It is theoretically possible that the hæmoglobin requiring bacilli represent a group of micro-organisms containing distinct species.

6. The occurrence of the Pfeiffer bacillus both in measles and in influenza constitutes suggestive evidence against its etiologic relationship in either disease. This evidence would be materially strengthened provided the identity of the strains from these two sources were accurately established.

7. The evidence which is available at present is not sufficiently complete to exclude the specific etiologic rôle of the Pfeiffer bacillus in some of the acute respiratory diseases.

* Neisser [13] reports that the Pfeiffer bacillus can be cultivated indefinitely on plain agar in "symbiosis" with ordinary micrococci.

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THE REACTION OF THE LEUCOCYTES IN EPIDEMIC INFLUENZA

By Beverly Douglas

During the epidemic of influenza occurring in the fall of 1918, the presence of a leucopenia in practically all the cases was commented on by numerous observers. An opportunity was afforded during the course of the epidemic in The Johns Hopkins Hospital to make rather extensive and careful studies of the blood, which it seems worth while to put on record. The data can best be presented in the form of tables, with notes appended thereto.

1. *General Course of the Leucocyte Curve in Uncomplicated Epidemic Influenza.*—It seemed that information of most value about the general course of the leucocyte curve could be obtained by compiling the averages of counts made in a number of cases on various days. The results of such compilations are recorded in Table I and graphically represented in Chart 1. The following points may be commented on:

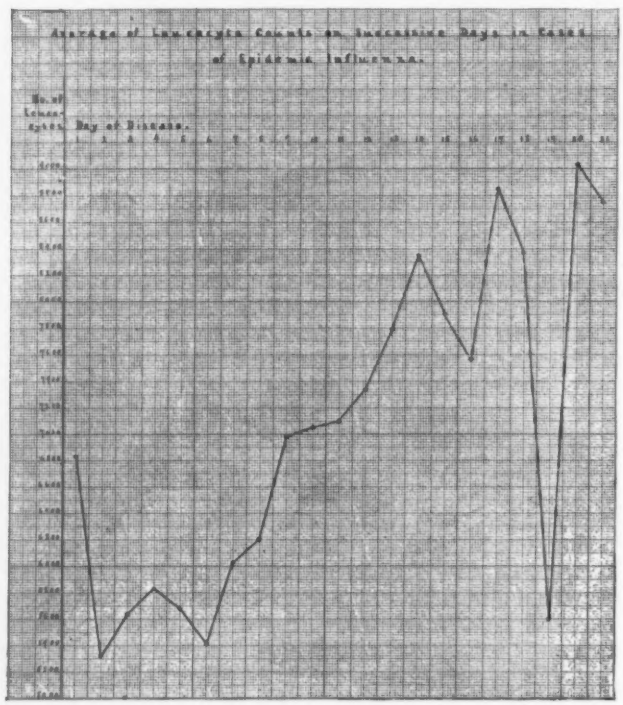

Chart 1.

Leucopenia is present, even on the day of onset of symptoms, the count falling somewhat on the following day and then gradually rising. One might almost imagine that there is some initial shock which depresses the leucocytes and from which they gradually escape during the subsequent days of the disease. It is also noteworthy that the return to normal count comes gradually, the average count still being subnormal on the twenty-first day.

Table I also shows the reaction of the leucocytes in the cases of influenza complicated by bronchopneumonia. These counts bear out the impression obtained at the time of the epidemic that a complicating bronchopneumonia, as a rule, does not lead to leucocytosis. An attempt was made to see if

TABLE I.—AVERAGE LEUCOCYTE COUNTS COMPILED FROM HISTORIES OF 250 EPIDEMIC INFLUENZA AND SUSPECTED INFLUENZA PATIENTS

Epidemic of Oct.-Nov., 1918. J. H. H.

	Flu	Acute resp. not flu	Afebrile flu	Flu & bronchopneu. fatal	Flu & bronchopneu. not fatal and fatal	Flu & bronchopneu. not fatal
3 or more days before onset.				3	7	4
				3127	3430	3656
2 days before onset.				1	5	4
				2625	4580	4818
Day before onset.				2	5	3
				4705	4010	3880
Day of onset.....	25[1]	4	1	6[2]	16[2]	10[2]
	6833	13120	5800	4250	4204	4175
2d	48	7	°	4	15	11
	5323	8130	....	5440	5408	5396
3d	41	5	2	4	15	11
	5644	7054	6700	6260	5564	5305
4th	33	°	1	2	10	8
	5838	....	5760	3280	5850	6492
5th	18	1	°	3	11	8
	5681	8800	....	8550	8644	8677
6th	31	1	1			7
	5412	8000	5200			7530
7th	13	1	1			4
	6036	4850	9450			8860
8th	28	7	3			4
	6203	8450	6910			13960
9th	14					5
	6985					9740
10th	21					5
	7050					8432
11th	17					4
	7100					7810
12th	25					4
	7340					10860
13th	25					2
	7790					9340
14th	25					1
	8350					9800
15th	23					4
	7900					7630
16th	12					1
	7560					8120
17th	15					
	8850					
18th	7					
	8370					
19th	2					
	5600					
20th	6					
	9030					
21st	5					
	8752					
22 days or more..	2					
	5230					
Grand average ...	436			25	126	174[3]
	6673			5040	6745	7066

[1] Index number=number of counts averaged for that day.
[2] Onset of pneumonia.
[3] All except uncomplicated flu.

the severity of the case from the clinical standpoint could be correlated with the extent of the leucopenia. For this purpose the cases were divided into mild, moderate and severe.

Counts made on or before the fourth day after onset showed in the mild cases an average of 5500, in the moderate, an average of 5800, and in the severe, an average of 5400. The lowest single count observed—one of 1900 cells, was in an extremely mild case. It seems, therefore, that in a general way, the extent of the leucopenia does not vary with the severity of the disease.

TABLE II.—SUPPLEMENTARY TABLE SHOWING HIGHEST AND LOWEST COUNTS ON EACH DAY. (See No. 1)

INFLUENZA EPIDEMIC OCT.-NOV., 1918. J. H. H.

	Flu	Acute resp. not flu	Afebrile flu	Flu c broncho-pneum. fatal	Flu c broncho-pneum. non-fatal
3 or more days before onset			Too Few.	4960	4960
				2000	2640
2 days before onset				2625	6000
				2625	3800
Day before onset				4760	5050
				4650	3140
Day of onset	10700	22480		6400	6400
	3440	6000		3400	2675
2d	10440	9200		10200	13000
	2500	7000		2460	2440
3d	11720	10000		14600	13100
	2640	4800		2600	2075
4th	12000	°°°°		3320	18400
	2240	°°°°		3240	2320
5th	12500	8800		18200	31360
	2520	8800		2200	2360
6th	10600	8000			11900
	3000	8000			4650
7th	10300	4850			14480
	2400	4850			5760
8th	10100	11980			24720
	2120	5440			8160
9th	11500				11920
	3260				6120
10th	12000				10200
	2640				6300
11th	12640				9800
	4720				3640
12th	14600				13120
	3100				8640
13th	10600				10280
	5200				8400
14th	16000				9800
	2500				9800
15th	13440				8750
	4000				5080
16th	10600				8120
	3320				8120
17th	30800				
	4480				
18th	14600				
	6250				
19th	7000				
	4040				
20th	14600				
	5080				
21st	12500				
	7100				
22d					

2. In Table II, which should be studied in connection with Table I, are presented the highest and lowest individual counts encountered in the various cases on each day of the disease. From this table, it is apparent that despite the general tendency to a leucopenia, there may in some cases be a moderate leucocytosis.

TABLE III.—AVERAGES OF PER CENT PMN. AND TOTAL LEUCOCYTE COUNTS COMPILED FROM 250 INFLUENZA HISTORIES IN J. H. H.

EPIDEMIC OF OCT.-NOV., 1918

Day of disease	Av. WBC	Av. PMN	Day of disease	Av. WBC	Av. PMN
Onset	4[1] 5220	72%	11th	3 6387	45%
2d	13 5575	66%	12th	6 7843	60%
3d	6 7900	59%	13th	3 8870	57%
4th	9 5675	53%	14th	6 7930	61%
5th	2 7350	61%	15th	11 7450	62%
6th	°°°	°°	16th	3 6550	69%
7th	1 6600	62%	17th	4 8330	62%
8th	3 6330	60%	18th	1 7400	79%
9th	5 6456	56%	19th	11 8080	73%
10th	4 9950	66%	20th	2 9870	47%

[1] Small index number=number of counts averaged.

TABLE IV.—INFLUENZA. VARIATION OF PERCENTAGE OF PMN. CELLS C. W. B. COUNTS (COUNTS AND DIFFERENTIALS ON SAME DAYS)

EPIDEMIC OF OCT.-NOV., 1918. J. H. H.

WBC	%PMN	WBC	%PMN	WBC	%PMN	WBC	%PMN	WBC	%PMN
3000-3500		4000-4500		5000-5500		6000-6500		7000-7500	
3000	40	4300	64	5040	68	6080	55	7000	75
3100	51	4440	75	5120	69	6080	66	7240	78
3160	57	4500	78	5260	70	6100	67	7400	78
3440	47	—	—	5440	53	6200	46?	7460	70
—	—	Av. 4413	69	5440	57	6300	80	—	—
Av. 3175	48.8	4500-5000		5480	61	—	—	Av. 7285	76
3500-4000		4520	40	Av. 5296	63	6320	81	7500-8000	
3560	34	4520	50			6440	62	7680	56
3640	69	4700	55	5500-6000		6480	58	7800	62
—	—	5000	53	5760	75	6500	67	7840	64
Av. 3600	51	—	—	5800	63	—	—	7920	47?
		Av. 4685	49.5	5880	56	Av. 6285	64.7	7960	52
				5880	46?	6500-7000		—	—
				5900	82	6600	63	Av. 7840	56
				—	—	6800	47?		
				Av. 5844	64.6	6800	68		
						6920	64		
						—	—		
						Av. 6780	60.5		
3000-4000		4000-5000		5000-6000		6000-7000		7000-8000	
Grand av. 3316	49.6	Av. 4750	57.8	Av. 5545	63.7	Av. 6404	61	Av. 7504	65

WBC	%PMN	WBC	%PMN	WBC	%PMN
8000-8500		9000-9500		10000 and above	
8000	68	9000	69	10000	68
8000	64	9040	67	10500	71
8040	72	9200	67	10600	57
8080	72	9200	68	10620	60
8160	60	9480	70	10880	60
8320	68	—	—	11500	60
8400	75	Av. 9184	68	11980	59
8440	56	9500-10000		—	—
—	—	None.		Av. 10870	62.3
Av. 8180	66.9			No differentials on WBC above this point.	
8500-9000					
8620	55				
8620	57				
8980	63				
Av. 8740	58.3				
8000-9000		9000-10000		10000-12000	
Grand av. 8332	64.5	9184	66.2	10995	62

3. Table III attempts to correlate the polymorphonuclear leucocytes with the total count. In this relatively small number of cases, no significant relationship is made out.

4. Table IV shows the relationship of the polymorphonuclears to the total count from another point of view—namely, the total count rather than the day of the disease.

Conclusions.—From these tabulations, the following general conclusions may be drawn:

Leucopenia is the rule in epidemic influenza, although a few cases may show normal counts or a slight leucocytosis.

The leucopenia frequently is present on the first day of the disease, after which it may become more marked for a few days, with a subsequent gradual tendency to rise until the normal mark is reached. In some cases the normal mark is overshot during convalescence, and leucocytosis may be present.

There is no constant relation between the leucocyte count and the severity of the disease.

The persistence of the leucopenia is the rule, even where bronchopneumonia, fatal or non-fatal, supervenes.

Differential counts show an absolute relative decrease in polymorphonuclear cells, as well as during the stage of leucopenia, varying to some extent with the total count.

Acute non-influenzal respiratory infections, as a rule, are accompanied by a leucocytosis.

A leucopenia is, therefore, a reliable diagnostic sign in epidemic influenza.

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Medical Report of the Durand Hospital of the John McCormick Institute for Infectious Diseases. For the First Five Years, March 12, 1913 to March 12, 1918. George H. Weaver, M. D., Physician-in-Charge. 1919. 4°. 32 pages. Chicago.

PUBLICATIONS

The following eight monographs:

Free Thrombi and Ball-Thrombi in the Heart. By J. H. Hewitt, M. D. 82 pages. Price, $1.00.

Benzol as a Leucotoxin. By Laurence Selling, M. D. 60 pages. Price, $1.00.

Primary Carcinoma of the Liver. By M. C. Winternitz, M. D. 42 pages. Price 75 cents.

The Statistical Experience Data of The Johns Hopkins Hospital, Baltimore, Md., 1892-1911. By Frederick L. Hoffman, LL.D., F.S.S. 161 pages. Price, $2.00.

The Origin and Development of the Lymphatic System. By Florence R. Sabin. 94 pages. Price, $2.00.

Venous Thrombosis During Myocardial Insufficiency. By Frank J. Sladen, M. D., and Milton C. Winternitz. M. D. Price, 75 cents.

Leukaemia of the Fowl: Spontaneous and Experimental. By Harry C. Schmeisser, M. D. Price, $2.00.

The Structure of the Normal Fibers of Purkinje in the Adult Human Heart and Their Pathological Alteration in Syphilitic Myocarditis. By O. Van Der Stricht and T. Wingate Todd. Price, $2.00.

are now on sale by The Johns Hopkins Press, Baltimore. Other monographs will appear from time to time

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